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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

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TITLE OF THE INVENTION (500 characters max)

METHODS FOR FABRICATING ISOLATED MICRO- AND NANO-STRUCTURES USING SOFT OR IMPRINT LITHOGRAPHY

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

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ENCLOSED APPLICATION PARTS ***(check all that apply)***

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[ ] Application Data Sheet. See 37 CFR 1.76

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[Page 1 of 2]

Respectfully submitted;

SIGNATURE ____

Date 08/27/04

REGISTRATION NO. 39,395 *(If appropriate)*

Docket Number: 421/110/2 PROV

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USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

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Docket Number 421/110/2 PROV

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Patty Wilson

Commissioner for Patents
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Re: U.S. Provisional Patent Application for
METHODS FOR FABRICATING ISOLATED MICRO- AND NANO-STRUCTURES USING SOFT OR IMPRINT LITHOGRAPHY
Our Ref. No. 421/110/2 PROV

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Please find enclosed the following:

1. Provisional Application Cover Sheet (2 pg.) in duplicate;

2. U.S. Provisional Patent Application (78 pgs.);

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Respectfully submitted,

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METHODS FOR FABRICATING ISOLATED MICRO- AND NANO-STRUCTURES USING SOFT OR IMPRINT LITHOGRAPHY

TECHNICAL FIELD

The presently disclosed subject matter describes a method of fabricating molecules for use in drug discovery and drug therapy; a method of patterning patterning natural and synthetic structures; a method of modifying the surface of an imprint lithography mold to impart surface characteristics to molded products; and improved ways to pattern structures and make isolated features.

SUMMARY

I. Method of Fabricating Molecules

In some embodiments, the presently disclosed subject matter describes methods and processes, and products by processes, for fabricating "molecules," for use in drug discovery and drug therapies. In some embodiments, the method or process for fabricating a molecule comprises a combinatorial method or process. In some embodiments, the method for fabricating molecules comprises a non-wetting imprint lithography (N-IL) method.

In some embodiments, the N-IL method further comprises a surface derived from or comprising a perfluoropolyether-containing material. In some embodiments, the N-IL method is used to generate isolated structures. In some embodiments, the isolated structures comprise isolated micro-structures. In some embodiments, the isolated structures comprise isolated nano-structures. In some embodiments, the isolated structures comprise a biodegradable material. In some embodiments, the isolated structures comprise a hydrophilic material. In some embodiments, the isolated structures comprise a hydrophobic

material. In some embodiments, the isolated structures comprise a particular shape. In some embodiments, the isolated structures further comprise "cargo."

In some embodiments, the N-IL method further comprises adding molecular modules, fragments, or domains to the solution to be molded. In some embodiments, the molecular modules, fragments, or domains impart functionality to the isolated structures. In some embodiments, the functionality imparted to the isolated structure comprises a therapeutic functionality.

In some embodiments, a therapeutic agent, such as a drug, is incorporated into the isolated structure. In some embodiments, the physiologically active drug is tethered to a linker to facilitate its incorporation into the isolated structure. In some embodiments, the domain of an enzyme or a catalyst is added to the isolated structure. In some embodiments, a ligand or an oligopeptide is added to the isolated structure. In some embodiments, the oligopeptide is functional. In some embodiments, the functional oligopeptide comprises a cell targeting peptide. In some embodiments, the functional oligopeptide comprises a cell penetrating peptide.

In some embodiments, a binder is added to the isolated structure. In some embodiments, the isolated structure comprising the binder is used to fabricate identical structures. In some embodiments, the isolated structure comprising the binder is used to fabricate structures of a varying structure. In some embodiments, the structures of a varying structure are used to explore the efficacy of a molecule as a therapeutic agent. In some embodiments, the method further comprises a method for drug discovery.

II. Method of Patterning Natural and Synthetic Structures

In some embodiments, the presently disclosed subject matter describes methods and processes, and products by processes, for generating surfaces and molds from natural structures, single molecules, or self-assembled structures. Accordingly, in some embodiments, the presently disclosed subject matter describes a method of patterning a natural structure, single molecule, and/or a self-assembled structure. In some embodiments, the method further comprises

replicating the natural structure, single molecule, and/or a self-assembled structure. In some embodiments, the method further comprises replicating the functionality of the natural structure, single molecule, and/or a self-assembled structure.

More particularly, in some embodiments, the method further comprises taking the impression or mold of a natural structure, single molecule, and/or a self-assembled structure. In some embodiments, the impression or mold is taken with a low surface energy polymeric precursor. In some embodiments, the low surface energy polymeric precursor comprises a perfluoropolyether (PFPE) functionally terminated di-acrylate. In some embodiments, the natural structure, single molecule, and/or self-assembled structure is selected from the group consisting of enzymes, viruses, micelles, and tissue surfaces.

In some embodiments, the impression or mold is used to replicate the features of the natural structure, single molecule, and/or a self-assembled structure into an isolated object or a surface. In some embodiments, a N-IL method is used to impart the features into a molded part or surface. In some embodiments, the molded part or surface produced by this process can be used in many applications, including, but not limited to, drug delivery, medical devices, coatings, catalysts, or mimics of the natural structures from which they are derived. In some embodiments, the natural structure comprises biological tissue. In some embodiments, the biological tissue comprises tissue from a bodily organ, such as a heart. In some embodiments, the biological tissue comprises vessels and bone. In some embodiments, the biological tissue comprises tendon or cartilage. For example, in some embodiments, the presently disclosed subject matter can be used to pattern surfaces for tendon and cartilage repair. Such repair typically requires the use of collagen tissue, which comes from cadavers and must be machined for use as replacements. Most of these replacements fail because one cannot lay down the primary pattern that is required for replacement. The soft lithographic methods described herein alleviate this problem.

In some embodiments, the presently disclosed subject matter can be applied to tissue regeneration using stem cells. Almost all stem cell approaches known in the art require molecular patterns for the cells to seed and then grow, thereby taking the shape of an organ, such as a liver, a kidney, or the like. In some embodiments, the molecular scaffold is cast and used as crystals to seed an organ in a form of transplant therapy. In some embodiments, the stem cell and nano-substrate is seeded into a dying tissue, e.g., liver tissue, to promote growth and tissue regeneration. In some embodiments, the material to be replicated in the mold comprises a material that is similar to or the same as the material that was originally molded. In some embodiments, the material to be replicated in the mold comprises a material that is different from and/or has different properties than the material that was originally molded. This approach could play an important role in addressing the organ transplant shortage.

In some embodiments, the presently disclosed subject matter is used to take the impression of one of an enzyme, a bacterium, and a virus. In some embodiments, the enzyme, bacterium, or virus is then replicated into a discrete object or onto a surface that has the shape reminiscent of that particular enzyme, bacterium, or virus replicated into it. In some embodiments, the mold itself is replicated on a surface, wherein the surface-attached replicated mold acts as a receptor site for an enzyme, bacterium, or virus particle. In some embodiments, the replicated mold is useful as a catalyst, a diagnostic sensor, a therapeutic agent, a vaccine, and the like. In some embodiments, the surface-attached replicated mold is used to facilitate the discovery of new therapeutic agents.

In some embodiments, the macromolecular, e.g., enzyme, bacterial, or viral, molded "mimics" serve as non-self-replicating entities that have the same surface topography as the original macromolecule, bacterium, or virus. In some embodiments, the molded mimics are used to create biological responses, e.g., an allergic response, to their presence, thereby creating antibodies or activating receptors. In some embodiments, the molded mimics function as a vaccine. In some embodiments, the efficacy of the biologically-active shape of the molded mimics is enhanced by a surface modification technique.

III. Method of Modifying the Surface of an Imprint Lithography Mold to Impart Surface Characteristics to Molded Products

In some embodiments, the presently disclosed subject matter describes a method of modifying the surface of an imprint lithography mold. In some embodiments, the method further comprises imparting surface characteristics to a molded product. In some embodiments, the molded product comprises an isolated molded product. In some embodiments, the isolate molded product is formed using a non-wetting imprint lithography technique. In some embodiments, the molded product comprises a contact lens, a medical device, and the like.

More particularly, the surface of a PFPE mold is modified by a surface modification step, wherein the surface modification step is selected from the group consisting of plasma treatment, chemical treatment, and the adsorption of molecules. In some embodiments, the molecules adsorbed during the surface modification step are selected from the group consisting of polyelectrolytes, poly(vinylalcohol), alkylhalosilanes, and ligands. In some embodiments, the structures, particles, or objects obtained from the surface-treated molds can be modified by the surface treatments in the mold. In some embodiments, the modification comprises the pre-orientation of molecules or moieties with the molecules comprising the molded products. In some embodiments, the pre-orientation of the molecules or moieties imparts certain properties to the molded products, including catalytic, wettable, adhesive, interactive, or not interactive, when the molded product is placed in another environment. In some embodiments, such properties are used to facilitate interactions with biological tissue or to prevent interaction with biological tissues. Applications of the presently disclosed subject matter include sensors, arrays, medical implants, medical diagnostics, disease detection, and separation media.

IV. Improved Ways to Pattern Structures and Make Isolated Features

In some embodiments, the presently disclosed subject matter describes a method of fabricating isolated liquid objects, the method comprising (a) contacting a liquid material with the surface of a first low surface energy material; (b) contacting the surface of a second low surface energy material with the liquid, wherein at least one of the surfaces of either the first or second low surface energy material is patterned; (c) sealing the surfaces of the first and the second low surface energy materials together; and (d) separating the two low surface energy materials to produce a replica pattern comprising liquid droplets.

In some embodiments, the liquid material comprises poly(ethylene glycol)-diacrylate. In some embodiments, the low surface energy material comprises perfluoropolyether-diacrylate. In some embodiments, a chemical process is used to seal the surfaces of the first and the second low surface energy materials. In some embodiments, a physical process is used to seal the surfaces of the first and the second low surface energy materials. In some embodiments, one of the surfaces of the low surface energy material is patterned. In some embodiments, one of the surfaces of the low surface energy material is not patterned.

In some embodiments, the method further comprises using the replica pattern composed of liquid droplets to fabricate other objects. In some embodiments, the replica pattern of liquid droplets is formed on the surface of the low surface energy material that is not patterned. In some embodiments, the liquid droplets undergo direct or partial solidification. In some embodiments, the liquid droplets undergo a chemical transformation. In some embodiments, the solidification of the liquid droplets or the chemical transformation of the liquid droplets produce freestanding objects. In some embodiments, the freestanding objects are harvested. In some embodiments, the freestanding objects are bonded in place. In some embodiments, the freestanding objects are directly solidified, partially solidified, or chemically transformed.

In some embodiments, the liquid droplets are directly solidified, partially solidified, or chemically transformed on the patterned surface to produce objects embedded in the mold. In some embodiments, the embedded objects are

harvested. In some embodiments, the embedded objects are bonded in place. In some embodiments, the embedded objects are used in other fabrication processes.

In some embodiments, the replica pattern of liquid droplets is transferred to other surfaces. In some embodiments, the transfer takes place before the solidification or chemical transformation process. In some embodiments, the transfer takes place after the solidification or chemical transformation process. In some embodiments, the surface to which the replica pattern of liquid droplets is transferred is selected from the group consisting of a non-low surface energy surface, a low surface energy surface, a functionalized surface, and a sacrificial surface. In some embodiments, the method produces a pattern on a surface that is essentially free of one or more scum layers. In some embodiments, the method is used to fabricate semiconductors and other electronic and photonic devices or arrays. In some embodiments, the method is used to create freestanding objects. In some embodiments, the method is used to create three-dimensional objects using multiple patterning steps. In some embodiments, the isolated or patterned object comprises materials selected from the group consisting of organic, inorganic, polymeric, and biological materials. In some embodiments, a surface adhesive agent is used to anchor the isolated structures on a surface.

In some embodiments, the liquid droplet arrays or solid arrays on patterned or non-patterned surfaces are used as regiospecific delivery devices or reaction vessels for additional chemical processing steps. In some embodiments, the additional chemical processing steps are selected from the group consisting of printing of organic, inorganic, polymeric, biological, and catalytic systems onto surfaces; synthesis of organic, inorganic, polymeric, biological materials; and other applications in which localized delivery of materials to surfaces is desired. Applications of the presently disclosed subject matter include, but are not limited to, micro and nanoscale patterning or printing of materials. In some embodiments, the materials to be patterned or printed are selected from the group consisting of surface-binding molecules, inorganic

compounds, organic compounds, polymers, biological molecules, nanoparticles, viruses, biological arrays, and the like.

In some embodiments, the applications of the presently disclosed subject matter include, but are not limited to, the synthesis of polymer brushes, catalyst patterning for CVD carbon nanotube growth, cell scaffold fabrication, the application of patterned sacrificial layers, such as etch resists, and the combinatorial fabrication of organic, inorganic, polymeric, and biological arrays.

In some embodiments, non-wetting imprint lithography, and related techniques, are combined with methods to control the location and orientation of chemical components within an individual object. In some embodiments, such methods improve the performance of an object by rationally structuring the object so that it is optimized for a particular application. In some embodiments, the method comprises incorporating biological targeting agents into particles for drug delivery, vaccination, and other applications. In some embodiments, the method comprises designing the particles to include a specific biological recognition motif. In some embodiments, the biological recognition motif comprises biotin/avidin and/or other proteins.

In some embodiments, the method comprises tailoring the chemical composition of these materials and controlling the reaction conditions, whereby it is then possible to organize the biorecognition motifs so that the efficacy of the particle is optimized. In some embodiments, the particles are designed and synthesized so that recognition elements are located on the surface of the particle in such a way to be accessible to cellular binding sites, wherein the core of the particle is preserved to contain bioactive agents, such as therapeutic molecules. In some embodiments, a non-wetting imprint lithography method is used to fabricate the objects, wherein the objects are optimized for a particular application by incorporating functional motifs, such as biorecognition agents, into the object composition. In some embodiments, the method further comprises controlling the microscale and nanoscale structure of the object by using methods selected from the group consisting of self-assembly, stepwise fabrication procedures, reaction conditions, chemical composition, crosslinking,

branching, hydrogen bonding, ionic interactions, covalent interactions, and the like. In some embodiments, the method further comprises controlling the microscale and nanoscale structure of the object by incorporating chemically organized precursors into the object. In some embodiments, the chemically organized precursors are selected from the group consisting of block copolymers and core-shell structures.

In sum, the presently disclosed subject matter describes a non-wetting imprint lithography technique that is scalable and offers a simple, direct route to such particles without the use of self-assembled, difficult to fabricate block copolymers and other systems.

DESCRIPTION
METHODS FOR FABRICATING ISOLATED MICRO- AND NANO-STRUCTURES USING SOFT OR IMPRINT LITHOGRAPHY

The concepts described herein include the methods and processes, and products by processes, to fabricate "molecules", perhaps in a combinatorial way, for use in drug discovery and drug therapies and to generate surfaces and ultimately molds from naturally occurring or biological substrates, molecules or assemblies of molecules. A further continuing concept is to pattern biological structures in an effort to replicate them or their functionality. One can also surface modify an imprint lithography mold to impart surface characteristics to Molded Products, where the molded products could be isolated molded products, such as those structures obtained using "Non-wetting Imprint Lithography", or can simply be the surface of a molded product, such as a contact lens, medical device, etc. A further refining concept involves different ways to pattern and fabricate isolated structures.

Fabricated Molecules:

With non-wetting imprint lithography (N-IL), especially as it pertains to N-IL where the surface is derived or comprised of perfluoropolyether-containing materials, one can generate very small isolated structures. These small isolated structures can be comprised of almost any materials including biodegradable, hydrophilic, or hydrophobic materials. These isolated structures can be of almost any shape and contain almost any cargo. One can add molecular modules, fragments or domains to a solution to be molded. These molecular modules, fragments or domains can impart functionality to the structures, including therapeutic functionality to the isolated structures derived from this process. For example one can add a drug, the drug could be tethered to a linker to facilitate it's incorporation into the structures. One could add a domain of an enzyme or a catalyst. One could add a ligand, or an oligopeptide. Such oligopeptides could be functional, like cell targeting peptides or cell penetrating peptides. Perhaps one could add a binder. One could do this in an attempt to make identical structures or structures that vary in some way to explore the efficacy of these molecules as therapeutics (i.e. a method for drug discovery).

Patterning of Natural and Synthetic Structures.

With a low surface energy polymeric precursor, such as a perfluoropolyether (PFPE) functionally terminated di-acrylate, one can take the impression or mold of natural structures, single molecules or self-assembled structures. These natural structures, single molecules or self-assembled structures can be enzymes, viruses, micelles, tissue surfaces, proteins, DNA, RNA, cells, organs, crystals, quasicrystals, carbon nanotubes, bucky balls, polymers, nanowires, polysaccharides, etc. Once impressions or molds of these structures or other self-assembled structures have been made with the low surface energy polymeric precursor, such as PFPE diacrylate, then the molds can be used to replicate those features into an isolated object or a surface, perhaps using non-wetting imprint lithographic methods. The molded parts or surfaces derived from the impressions or molds can be used in many applications including drug delivery, medical devices, coatings, catalysts, or mimics of the biological structures that they were derived from, protective gear, membranes. For example, one can take the impression or make a mold of a biological heart tissue using PFPE Diacrylate. Then use that mold to make a replicate of the biological tissue out of a wide range of materials. Another example would be the patterning of surfaces for tendon and cartilage repair. Such repair typically requires the use of collagen polymer. These tissues come from cadavers and they need to be machined

for use as replacements. Most of these replacements fail because one can not lay down the primary pattern that is required for attachment. This can be alleviated using the soft lithographic methods described herein. This approach can also be used for other tissues such as vessels and bone. Another example would be related to tissue regeneration using stem cells. Almost all stem cell approaches require molecular patterns for the cells to seed and than grow taking on the shape of an organ such as liver, kidney, etc. One could cast these molecular scaffolds and use these as crystals to seed an organ in a form of transplant therapy. In this case, the stem cell and nano substrate would be seeded into a dying tissue (e.g. liver), and in that environment, promote growth and tissue regeneration. This could play an important role in solving the organ transplant shortage. The material chosen to be replicated in the mold can be a material that is similar or the same as the material that was originally molded or from a completely different material with different properties.

One could also take the impression of an enzyme, or bacteria or virus. Then one could replicate the enzyme, bacteria or virus into a discrete object or onto a surface that has the shape reminiscent of that enzyme, bacteria or virus replicated into it. Or one could replicate the mold itself on a surface to act as a receptor site for an enzyme, bacteria or virus particle. Such materials could be useful as catalysts, diagnostic sensors, therapeutics, vaccines, etc. The latter surfaces maybe useful in the discovery of new therapeutics. The former molded macromolecular, bacterial or virus "mimics" could serve as non-self-replicating entities that have the same surface topography as the original macromolecule, bacteria or virus. And insofar as molded "mimic" shape promotes a biological response, said mimics might be used to create (allergic?) biological responses to their presence creating antibodies or activating receptors and thereby function as a vaccine via induction of said Reponses. The efficacy of the biologically-active shape of such molded mimics might be further enhanced by surface modification methods described below.

Surface Modify an Imprint Lithography Mold to Impart Surface Characteristics to Molded Products

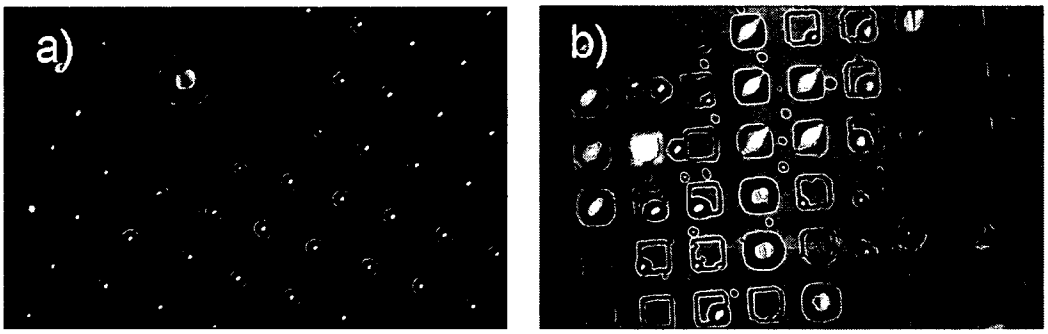
With a PFPE mold, one can modify its surface, by any number of methods, which can influence the material to be molded within it. Such surface modification steps could include plasma treatment, chemical treatment, adsorption of molecules (such as polyelectrolytes, poly(vinylalcohol), alkylchlorosilanes, ligands, etc). The structures, particles or objects obtained from these surface treated molds can be modified by the surface treatments in the mold. Such modifications can include pre-orientation of molecules or moieties within molecules comprising the molded products. Such orientations make the molded products catalytic, wettable, adhesive, interactive or not interactive when the molded product is place in another environment. Such properties could also be used to facilitate interactions with biological tissue or prevent interaction with biological tissues. Such applications could include sensors, arrays, medical implants, medical diagnostics, disease detection, and separation media.

Improved ways to Pattern Structures and make Isolated Features:

In addition to molding a liquid precursor between two chemically similar low surface energy materials where one of these materials is patterned, it is also possible to exploit the non-wetting capability of low-energy surfaces to fabricate isolated or patterned objects using extended methods. For example, a similar molding process can be used to fabricate isolated liquid objects within a low surface energy matrix by molding the liquid between two low surface energy materials and sealing the surfaces together using a physical or

chemical process. After separation of the two low surface energy materials, a replica pattern consisting of liquid droplets that is similar to the pattern of the original master may exist on the patterned and/or the non-patterned surface. This replica composed of liquid droplets can then be used for further fabrication of other objects. Figure 1a shows a patterned array of liquid PEG-diacrylate droplets, deposited on a non-patterned PFPE-diacrylate surface, that were formed by molding a liquid precursor against a patterned PFPE-diacrylate elastomer. Figure 1b shows an array of liquid droplets, formed in a patterned PFPE-diacrylate elastomer, by molding a liquid precursor against a non-patterned PFPE-diacrylate surface.

Additional fabrication steps using replicas composed of liquid droplets can include direct or partial solidification or other chemical transformation of the liquid precursor on the non-patterned surface to produce freestanding objects which could be harvested or bonded in place, or direct solidification, partial solidification, or other chemical transformation of the precursor on the patterned surface to produce objects embedded in the mold which could be harvested, bonded in place, or used in other fabrication processes. Alternatively, one can transfer the patterned liquid droplets to other surfaces before or after solidification / chemical transformation to replicate isolated or patterned objects onto other surfaces, including but not limited to: non-low surface energy, low surface energy, functionalized, and sacrificial surfaces. This method could be useful to create patterns on surface that are essentially free of scum layers and as such can be useful in the fabrication of semiconductors, and other electronic and photonic devices or arrays. Similar methods can also be used to create freestanding objects or to create three dimensional objects using multiple patterning steps.[1] As an example of this fabrication strategy, figure 2 shows isolated poly(PEG-diacrylate) particles on a glass surface that were formed by polymerization of a patterned, liquid droplet array against a glass surface. After fabrication, structures of this type could be harvested as isolated objects or bonded in place to create surface-bound structures. This fabrication methodology can be used to create isolated or patterned objects consisting of organic, inorganic, polymeric and/or biological materials. Surface adhesive agents could also be used to anchor the isolated structures on a surface.



Liquid droplet arrays or solid arrays on patterned or non-patterned surfaces can be used as regiospecific reactant delivery devices or reaction vessels for additional chemical processing steps, including but not limited to: printing of organic, inorganic, polymeric, biological, or catalytic systems onto surfaces, synthesis of organic, inorganic, polymeric, or biological materials, and other applications where localized delivery of materials to surfaces is desired. Applications for this type of technology include micro or nanoscale patterning or printing of materials such as surface-binding molecules, inorganic compounds, organic compounds, polymers, biological molecules,

nanoparticles, viruses, biological arrays, etc. Other applications include synthesis of polymer brushes, catalyst patterning for CVD carbon nanotube growth, cell scaffold fabrication, the application of patterned sacrificial layers such as etch resists, or the combinatorial fabrication of organic, inorganic, polymeric, or biological arrays.

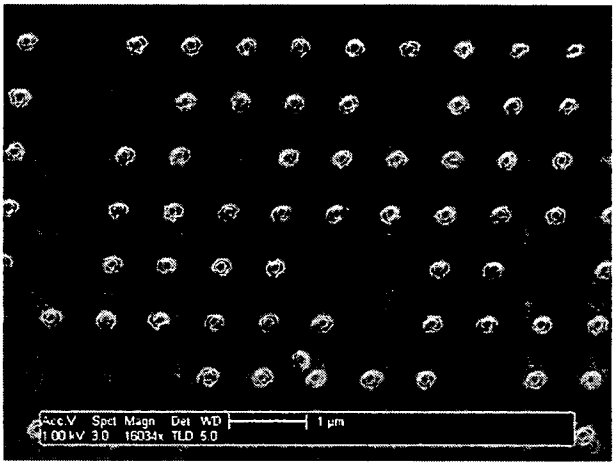


Figure 2. Isolated poly(PEG-diacrylate) particles on glass surfaces, fabricated using a liquid droplet array

Non-wetting imprint lithography and related techniques can be combined with methods to control the location and orientation of chemical components within an individual object. These methods can improve the performance of an object by rationally structuring it so that the object is optimized for a particular application. For example, it is possible to incorporate biological targeting agents into particles for drug delivery, vaccination and other applications by designing the particles to include a specific biological recognition motif, such as biotin/avidin and other proteins. Furthermore, by tailoring the chemical composition of these materials and controlling the reaction conditions, it is possible to organize these biorecognition motifs so that the efficacy of the particle is optimized; for example, the particles can be designed and synthesized so that recognition elements are located on the surface of the particle where they are accessible to cellular binding sites, while the core of the particle is preserved to contain bioactive agents such as therapeutic molecules.[ii] Similarly, objects fabricated using non-wetting imprint lithography can be optimized for a particular application by incorporating functional motifs, such as biorecognition agents, into the object composition, and by controlling the microscale and nanoscale structure of the object using self-assembly, stepwise fabrication procedures, reaction conditions, chemical composition, cross-linking, branching, hydrogen bonding, ionic interactions, covalent interactions etc., and by incorporating chemically organized precursors, such as block copolymers or core-shell structures, into the objects. N-IL processes are scalable and offer a simple, direct route to such particles without the use of self-assembled, difficult to fabricate block copolymers and other systems[2].

1. Zhao X. M, Xia Y., and Whitesides, G. M., *Advanced Materials* **1996**, 8, 10, 837-840.
[ii] Oi, K., Ma, Q., Remsen, E. E., Clark, C. G., and Wooley, K. M., *Journal of the American Chemical Society* **2004**, 126, 21, 6599-6607.

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Determination of the Bioavailability of Biotin Conjugated onto Shell Cross-Linked (SCK) Nanoparticles

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Abstract: Shell cross-linked nanoparticles (SCKs) presenting surface- and bioavailable biotin functional groups were synthesized via a mixed micelle methodology, whereby co-micellization of chain terminal biotinylated poly(acrylic acid)-*b*-poly(methyl acrylate) (PAA-*b*-PMA) and nonbiotinylated PAA-*b*-PMA were cross-linked in an intramicellar fashion within the shell layer of the mixed micelles, between the carboxylic acid groups of PAA and the amine functionalities of 2,2′-(ethylenedioxy)diethylamine. The hydrodynamic diameters (D_h) of the micelles and the SCKs with different biotinylated block copolymer contents were determined by dynamic light scattering (DLS), and the dimensions of the SCKs were characterized with tapping-mode atomic force microscopy (AFM) and transmission electron microscopy (TEM). The amount of surface-available biotin was tuned by varying the stoichiometric ratio of the biotinylated PAA-*b*-PMA versus the nonbiotinylated PAA-*b*-PMA, as demonstrated with solution-state, binding interaction analyses, an avidin/HABA (avidin/4′-hydroxyazobenzene-2-carboxylic acid) competitive binding assay, and fluorescence correlation spectroscopy (FCS). The avidin/HABA assay found the amount of available biotin at the surface of the biotinylated SCK nanoparticles to increase with increasing biotin-terminated block copolymer incorporation, but to be less than 25% of the theoretical value. FCS measurements showed the same trend.

Introduction

Functionalization of nanoparticles enables tuning of the interactions among themselves as well as with their surrounding environment, by which controlled formation of supramolecular architectures can be achieved.[1-10] These nanostructured materials have potential applications for the construction of devices with unique optical,[11-15] electronic,[16-19] magnetic,[20-22] and catalytic properties.[23-26] Moreover, there has been ever-growing interest in preparing nanoparticles conjugated with biomolecules[27,28] for biomimetics,[29-31] targeted delivery,[32-39] bio-

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(1) Hamley, I. W. *Angew. Chem., Int. Ed.* **2003**, *42*, 1692−1712.
(2) Cölfen, H.; Mann, S. *Angew. Chem., Int. Ed.* **2003**, *42*, 2350−2365.
(3) Shenhar, R.; Rotello, V. M. *Acc. Chem. Res.* **2003**, *36*, 549−561.
(4) Whitesides, G. M.; Grzybowski, B. *Science* **2002**, *295*, 2418−2421.
(5) Whitesides, G. M.; Boncheva, M. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 4769−4774.
(6) Förster, S.; Plantenberg, T. *Angew. Chem., Int. Ed.* **2002**, *41*, 688−714.
(7) Shipway, A. N.; Willner, I. *Chem. Commun.* **2001**, 2035−2045.
(8) Shipway, A. N.; Katz, E.; Willner, I. *ChemPhysChem* **2000**, *1*, 18−52.
(9) Hernandez-Lopez, J. L.; Bauer, R. E.; Chang, W. S.; Glasser, G.; Grebel-Koehler, D.; Klapper, M.; Kreiter, M.; Leclaire, J.; Majoral, J. P.; Mittler, S.; Müllen, K.; Vasilev, K.; Weil, T.; Wu, J.; Zhu, T.; Knoll, W. *Mater. Sci. Eng., C* **2003**, *C23*, 267−274.
(10) Storhoff, J. J.; Mirkin, C. A. *Chem. Rev.* **1999**, *99*, 1849−1862.
(11) Gerion, D.; Pinaud, F.; Williams, S. C.; Parak, W. J.; Zanchet, D.; Weiss, S.; Alivisatos, A. P. *J. Phys. Chem. B* **2001**, *105*, 8861−8871.
(12) Mattoussi, H.; Mauro, J. M.; Goldman, E. R.; Green, T. M.; Anderson, G. P.; Sundar, V. C.; Bawendi, M. G. *Phys. Status Solidi B* **2001**, *224*, 277−283.
(13) Murray, C. B.; Kagan, C. R.; Bawendi, M. G. *Annu. Rev. Mater. Sci.* **2000**, *30*, 545−610.
(14) Elghanian, R.; Storhoff, J. J.; Mucic, R. C.; Letsinger, R. L.; Mirkin, C. A. *Science* **1997**, *277*, 1078−1080.
(15) Holtz, J. H.; Asher, S. A. *Nature* **1997**, *389*, 829−832.
(16) Milliron, D. J.; Alivisatos, A. P.; Pitois, C.; Edder, C.; Fréchet, J. M. J. *Adv. Mater.* **2003**, *15*, 58−61.
(17) Coe, S.; Woo, W.-K.; Bawendi, M.; Bulović, V. *Nature* **2002**, *420*, 800−803.
(18) Park, S.-J.; Lazarides, A. A.; Mirkin, C. A.; Brazis, P. W.; Kannewurf, C. R.; Letsinger, R. L. *Angew. Chem., Int. Ed.* **2000**, *39*, 3845−3848.
(19) Anicet, N.; Bourdillon, C.; Moiroux, J.; Savéant, J.-M. *J. Phys. Chem. B* **1998**, *102*, 9844−9849.
(20) Wang, X.-S.; Arsenault, A.; Ozin, G. A.; Winnik, M. A.; Manners, I. *J. Am. Chem. Soc.* **2003**, *125*, 12686−12687.
(21) Ginzburg, M.; MacLachlan, M. J.; Yang, S. M.; Coombs, N.; Coyle, T. W.; Raju, N. P.; Greedan, J. E.; Herber, R. H.; Ozin, G. A.; Manners, I. *J. Am. Chem. Soc.* **2002**, *124*, 2625−2639.
(22) Yan, X.; Liu, G.; Liu, F.; Tang, B. Z.; Peng, H.; Pakhomov, A. B.; Wong, C. Y. *Angew. Chem., Int. Ed.* **2001**, *40*, 3593−3596.
(23) Bosman, A. W.; Vestberg, R.; Heumann, A.; Fréchet, J. M. J.; Hawker, C. J. *J. Am. Chem. Soc.* **2003**, *125*, 715−728.
(24) Hecht, S.; Fréchet, J. M. J. *Angew. Chem., Int. Ed.* **2001**, *40*, 74−91.
(25) Piotti, M. E.; Rivera, F., Jr.; Bond, R.; Hawker, C. J.; Fréchet, J. M. J. *J. Am. Chem. Soc.* **1999**, *121*, 9471−9472.
(26) Klingelhöfer, S.; Heitz, W.; Greiner, A.; Oestreich, S.; Förster, S.; Antonietti, M. *J. Am. Chem. Soc.* **1997**, *119*, 10116−10120.
(27) Niemeyer, C. M. *Angew. Chem., Int. Ed.* **2001**, *40*, 4128−4158.
(28) Narain, R.; Armes, S. P. *Macromolecules* **2003**, *36*, 4675−4678.
(29) Sarikaya, M.; Tamerler, C.; Jen, A. K. Y.; Schulten, K.; Baneyx, F. *Nat. Mater.* **2003**, *2*, 577−585.
(30) Thibault, R. J., Jr.; Galow, T. H.; Turnberg, E. J.; Gray, M.; Hotchkiss, P. J.; Rotello, V. M. *J. Am. Chem. Soc.* **2002**, *124*, 15249−15254.
(31) Mann, S. *Angew. Chem., Int. Ed.* **2000**, *39*, 3392−3406.
(32) Choi, S.-W.; Kim, W.-S.; Kim, J.-H. *J. Dispersion Sci. Technol.* **2003**, *24*, 475−487.
(33) Berry, C. C.; Curtis, A. S. G. *J. Phys. D: Appl. Phys.* **2003**, *36*, R198−R206.
(34) Kakizawa, Y.; Kataoka, K. *Adv. Drug Delivery Rev.* **2002**, *54*, 203−222.

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detection,[40-44] diagnosis, and treatment purposes,[33,45-49] each of which depends on the biomolecule nanoparticle conjugates remaining bioactive and bioavailable after conjugation.

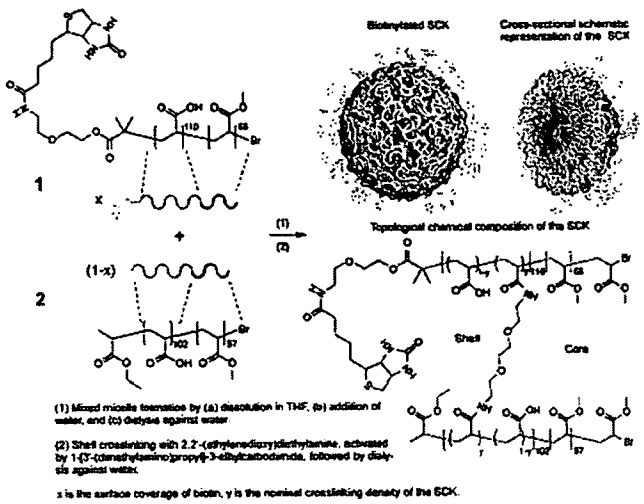
Shell cross-linked nanoparticles (SCKs),[50-58] a class of well-defined, polymeric, nanostructured materials with a hydrophobic core domain and a hydrophilic shell layer, have received recent attention as biocompatible[59] and stable[60] nanoscale scaffolds from which bioactive elements can be presented.[61-64] The general methodology that has been developed for the preparation of SCKs involves the supramolecular assembly of block copolymers into polymer micelles,[65-68] followed by covalent cross-linking reactions throughout the shell layer. Synthetic strategies for surface functionalization of SCKs have also been established,[69] based upon mixed micelle formation[70,71] or postpreparation functionalization reactions.[64] In each case, the determination of the surface- and bioavailability of the functional groups covalently linked within the hydrogel-like shell layer of the SCKs remains challenging, due to the interplay of the confinement versus flexibility of the partially crosslinked polymer chains constituting the SCK shell,[72,73] the mobility of the entire nanostructure,[74,75] and the composition of the functional groups.

The interaction of biotin and avidin as a ligand−receptor pair, widely used in the field of biology and medicine for purification, localization, and diagnostics, has served as a well-defined model system to probe bioavailability.[76,77] Applications utilizing protein recognition of biotinylated species, including small molecules, polymers, lipids, nucleic acids, proteins, and nanoparticles, have been extended to fabricate novel nanoscopic assemblies, such as two-dimensional arrays of biotin−avidin conjugates,[78] protein−polymer amphiphiles,[79] protein−lipid monolayers,[80] protein multilayers,[81] protein−polymer multilayers,[82] protein−DNA multilayers,[19,83] protein−nanoparticle composites,[84-87] and functionalized surfaces,[88-92] among others. Biotinylated nanoparticles,[93-95] and microparticles[96,97] with sizes ranging from globular proteins to cells, have been utilized as model systems to study and mimic the multivalent interactions[30,98-100] that occur in protein−cell recognition and cell−cell adhesion processes.[96,101]

Given the high application potential and fundamental significance of biotinylated nanoparticles, the synthesis and study of biotinylated SCKs were undertaken. Interest in these materials

(35) Kataoka, K.; Harada, A.; Nagasaki, Y. *Adv. Drug Delivery Rev.* **2001**, *47*, 113−131.
(36) Langer, R. *Science* **2001**, *293*, 58−59.
(37) Lynn, D. M.; Amiji, M. M.; Langer, R. *Angew. Chem., Int. Ed.* **2001**, *40*, 1707−1710.
(38) Langer, R. *Nature* **1998**, *392*, 5−10.
(39) Savić, R.; Luo, L.; Eisenberg, A.; Maysinger, D. *Science* **2003**, *300*, 615−618.
(40) Nam, J.-M.; Thaxton, C. S.; Mirkin, C. A. *Science* **2003**, *301*, 1884−1886.
(41) Cao, Y. C.; Jin, R.; Mirkin, C. A. *Science* **2002**, *297*, 1536−1540.
(42) Taton, T. A.; Mirkin, C. A.; Letsinger, R. L. *Science* **2000**, *289*, 1757−1760.
(43) Willner, I.; Shipway, A. N.; Willner, B. *ACS Symp. Ser.* **2003**, *844*, 88−105.
(44) Velev, O. D.; Kaler, E. W. *Langmuir* **1999**, *15*, 3693−3698.
(45) Pankhurst, Q. A.; Connolly, J.; Jones, S. K.; Dobson, J. *J. Phys. D: Appl. Phys.* **2003**, *36*, R167−R181.
(46) Hamblett, K. J.; Kegley, B. B.; Hamlin, D. K.; Chyan, M.-K.; Hyre, D. E.; Press, O. W.; Wilbur, D. S.; Stayton, P. S. *Bioconjugate Chem.* **2002**, *13*, 588−598.
(47) Alivisatos, A. P. *Sci. Am.* **2001**, *285*, 67−73.
(48) Wilbur, D. S.; Pathare, P. M.; Hamlin, D. K.; Weerawarna, S. A. *Bioconjugate Chem.* **1997**, *8*, 819−832.
(49) Wilbur, S. D.; Hamlin, D. K.; Vessella, R. L.; Stray, J. E.; Buhler, K. R.; Stayton, P. S.; Klumb, L. A.; Pathare, P. M.; Weerawarna, S. A. *Bioconjugate Chem.* **1996**, *7*, 689−702.
(50) Wooley, K. L. *J. Polym. Sci., Part A: Polym. Chem.* **2000**, *38*, 1397−1407.
(51) Wooley, K. L. *Chem.−Eur. J.* **1997**, *3*, 1397−1399.
(52) Thurmond, K. B., II; Kowalewski, T.; Wooley, K. L. *J. Am. Chem. Soc.* **1996**, *118*, 7239−7240.
(53) Thurmond, K. B., II; Kowalewski, T.; Wooley, K. L. *J. Am. Chem. Soc.* **1997**, *119*, 6656−6665.
(54) Huang, H.; Wooley, K. L.; Remsen, E. E. *Chem. Commun.* **1998**, 1415−1416.
(55) Ding, J.; Liu, G. *Macromolecules* **1998**, *31*, 6554−6558.
(56) Bütün, V.; Billingham, N. C.; Armes, S. P. *J. Am. Chem. Soc.* **1998**, *120*, 12135−12136.
(57) Sanji, T.; Nakatsuka, Y.; Kitayama, F.; Sakurai, H. *Chem. Commun.* **1999**, 2201−2202.
(58) Cao, L.; Manners, I.; Winnik, M. A. *Macromolecules* **2001**, *34*, 3353−3360.
(59) Becker, M. L.; Joralemon, M. J.; Remsen, E. E.; Endres, P. J.; Cegelski, L.; Huang, H.; Schaefer, J.; Wooley, K. L. *Polym. Prepr.* **2002**, *43*, 682−683.
(60) Clark, C. G., Jr.; Wooley, K. L. *Curr. Opin. Colloid Interface Sci.* **1999**, *4*, 122−129.
(61) Thurmond, K. B., II; Remsen, E. E.; Kowalewski, T.; Wooley, K. L. *Nucleic Acids Res.* **1999**, *27*, 2966.
(62) Liu, J.; Zhang, Q.; Remsen, E. E.; Wooley, K. L. *Biomacromolecules* **2001**, *2*, 362−368.
(63) Becker, M. L.; Liu, J.; Wooley, K. L. *Chem. Commun.* **2003**, 802−803.
(64) Pan, D.; Turner, J. L.; Wooley, K. L. *Chem. Commun.* **2003**, 2400−2401.
(65) Discher, D. E.; Eisenberg, A. *Science* **2002**, *297*, 967−973.
(66) Zhang, L.; Eisenberg, A. *Science* **1995**, *268*, 1728−1731.
(67) Zhang, L.; Yu, K.; Eisenberg, A. *Science* **1996**, *272*, 1777−1779.
(68) Matějíček, P.; Uhlík, F.; Limpouchová, Z.; Procházka, K.; Tuzar, Z.; Webber, S. E. *Macromolecules* **2002**, *35*, 9487−9496.
(69) Becker, M. L.; Joralemon, M. J.; Liu, J.; Ma, Q.; Murthy, K. S.; Qi, K.; Remsen, E. E.; Zhang, Q.; Wooley, K. L. *Polym. Prepr.* **2002**, *43*, 323.
(70) Matějíček, P.; Humpolíčková, J.; Procházka, K.; Tuzar, Z.; Spírková, M.; Hof, M.; Webber, S. E. *J. Phys. Chem. B* **2003**, *107*, 8232−8240.
(71) Terreau, O.; Luo, L.; Eisenberg, A. *Langmuir* **2003**, *19*, 5601−5607.
(72) Huang, H.; Wooley, K. L.; Schaefer, J. *Macromolecules* **2001**, *34*, 547−551.
(73) O'Connor, R. D.; Zhang, Q.; Wooley, K. L.; Schaefer, J. *Helv. Chim. Acta* **2002**, *85*, 3219−3224.
(74) Huang, H.; Kowalewski, T.; Wooley, K. L. *J. Polym. Sci., Part A* **2003**, *41*, 1659−1668.
(75) Murthy, K. S.; Ma, Q.; Remsen, E. E.; Kowalewski, T.; Wooley, K. L. *J. Mater. Chem.* **2003**, *13*, 2785−2795.
(76) Wilchek, M.; Bayer, E. A. *Anal. Biochem.* **1988**, *171*, 1−32.
(77) Wilchek, M.; Bayer, E. A. *Methods Enzymol.* **1990**, *184*, 5−13.
(78) Wang, S.-W.; Robertson, C. R.; Gast, A. P. *Langmuir* **1999**, *15*, 1541−1548.
(79) Hannink, J. M.; Cornelissen, J. J. L. M.; Farrera, J. A.; Foubert, P.; De Schryver, F. C.; Sommerdijk, N. A. J. M.; Nolte, R. J. M. *Angew. Chem., Int. Ed.* **2001**, *40*, 4732−4734.
(80) Ahlers, M.; Mueller, W.; Reichert, A.; Ringsdorf, H.; Venzmer, J. *Angew. Chem.* **1990**, *102*, 1310−1327.
(81) Müller, W.; Ringsdorf, H.; Rump, E.; Wildburg, G.; Zhang, X.; Angermaier, L.; Knoll, W.; Liley, M.; Spinke, J. *Science* **1993**, *262*, 1706−1708.
(82) Anzai, J.-i.; Nishimura, M. *J. Chem. Soc., Perkin Trans. 2* **1997**, 1887−1889.
(83) Ijiro, K.; Ringsdorf, H.; Birch-Hirschfeld, E.; Hoffmann, S.; Schilken, U.; Strube, M. *Langmuir* **1998**, *14*, 2796−2800.
(84) Park, S.-J.; Lazarides, A. A.; Mirkin, C. A.; Letsinger, R. L. *Angew. Chem., Int. Ed.* **2001**, *40*, 2909−2912.
(85) Lala, N.; Sastry, M. *Phys. Chem. Chem. Phys.* **2000**, *2*, 2461−2466.
(86) Mann, S.; Shenton, W.; Li, M.; Connolly, S.; Fitzmaurice, D. *Adv. Mater.* **2000**, *12*, 147−150.
(87) Shenton, W.; Davis, S. A.; Mann, S. *Adv. Mater.* **1999**, *11*, 449−452.
(88) Faucher, K. M.; Sun, X.-L.; Chaikof, E. L. *Langmuir* **2003**, *19*, 1664−1670.
(89) Sun, X.-L.; Faucher, K. M.; Houston, M.; Grande, D.; Chaikof, E. L. *J. Am. Chem. Soc.* **2002**, *124*, 7258−7259.
(90) Lahann, J.; Balcells, M.; Rodon, T.; Lee, J.; Choi, I. S.; Jensen, K. F.; Langer, R. *Langmuir* **2002**, *18*, 3632−3638.
(91) Hyun, J.; Zhu, Y.; Liebmann-Vinson, A.; Beebe, T. P., Jr.; Chilkoti, A. *Langmuir* **2001**, *17*, 6358−6367.
(92) Hyun, J.; Chilkoti, A. *J. Am. Chem. Soc.* **2001**, *123*, 6943−6944.
(93) Gref, R.; Couvreur, P.; Barratt, G.; Mysiakine, E. *Biomaterials* **2003**, *24*, 4529−4537.
(94) Schroedter, A.; Weller, H. *Angew. Chem., Int. Ed.* **2002**, *41*, 3218−3221.
(95) Sastry, M.; Lala, N.; Patil, V.; Chavan, S. P.; Chittiboyina, A. G. *Langmuir* **1998**, *14*, 4138−4142.
(96) Cannizzaro, S. M.; Padera, R. F.; Langer, R.; Rogers, R. A.; Black, F. E.; Davies, M. C.; Tendler, S. J. B.; Shakesheff, K. M. *Biotechnol. Bioeng.* **1998**, *58*, 529−535.
(97) Gao, J.; Niklason, L.; Zhao, X.-M.; Langer, R. *J. Pharm. Sci.* **1998**, *87*, 246−248.
(98) Mammen, M.; Chio, S.-K.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2755−2794.
(99) Cairo, C. W.; Gestwicki, J. E.; Kanai, M.; Kiessling, L. L. *J. Am. Chem. Soc.* **2002**, *124*, 1615−1619.
(100) Boal, A. K.; Rotello, V. M. *J. Am. Chem. Soc.* **2000**, *122*, 734−735.
(101) Barrientos, A. G.; de la Fuente, J. M.; Rojas, T. C.; Fernández, A.; Penadés, S. *Chem.-Eur. J.* **2003**, *9*, 1909−1921.

Scheme 1



is based primarily upon their value in the determination of the surface- and bioavailability of functional groups that are incorporated into the SCK nanostructure via a mixed micelle methodology. The mixed micelle methodology, nominally, is a co-micellization process involving amphiphilic block copolymers, at least one of which contains a biotin unit as the hydrophilic chain terminus. It is expected that an advantage of the mixed micelle strategy is the ability to control the degree of surface coverage by the biotin functional groups, via control over the stoichiometric ratio of the chain end functionalized and nonfunctionalized, amphiphilic block copolymers. Although the placement of the functionality is at the hydrophilic chain end, the actual location of this functionality with respect to the SCK's surface will depend on many factors. These include conformations of the polymer chains, which are dependent upon the nature of the chain end functionality, the composition of the block copolymers, the conditions employed for micelle formation, and the reaction conditions during shell cross-linking.[102-104] It was hypothesized that at least a portion of the functional groups, which are biotin in this particular case, will be surface-exposed after both micellization and shell cross-linking. The results of the present study support this hypothesis based on detailed solution-state, binding interaction analyses, employing an avidin/HABA (avidin/4'-hydroxyazobenzene-2-carboxylic acid) competitive binding assay and fluorescence correlation spectroscopy (FCS).

Results and Discussion

The preparation of biotinylated SCK nanoparticles via the mixed micelle methodology involves a combination of co-micellization and covalent stabilization within the shell layer (Scheme 1). The block copolymer precursors were designed to be composed of similar hydrophobic and hydrophilic block segment compositions and lengths to provide a uniform distribution of the mixed polymer chains throughout the micelles.[105,106]

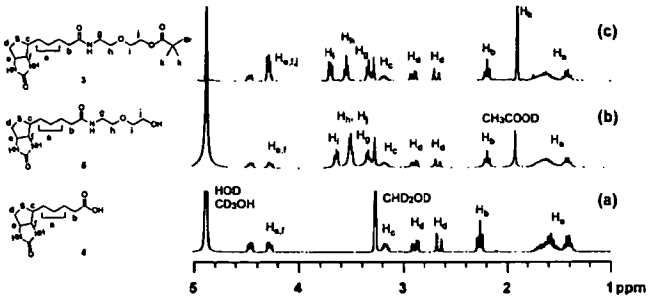
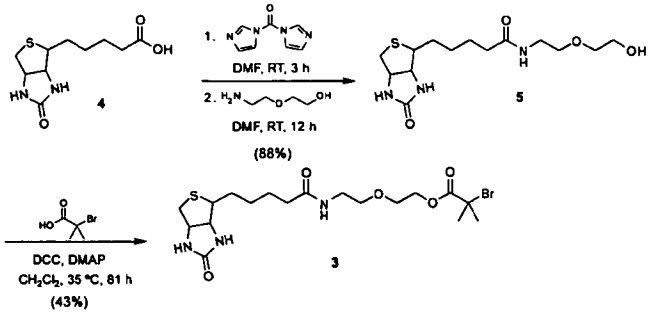


Figure 1. ^{1}H NMR spectra of (a) biotin, **4**, (b) biotinylated alcohol, **5**, and (c) biotinylated initiator, **3**, in CD_3OD.

Scheme 2



In addition, the hydrophilic block segment that carried the biotin unit was lengthened to enhance its presentation on the surfaces of the micelles and the corresponding SCKs. To further increase the solubility of the biotin functional group in aqueous media and to reduce interparticle aggregation, which could potentially result from the functionalized chain end, an ethylene oxide linker was used.[107] Therefore, amphiphilic diblock copolymers, poly(acrylic acid)-*b*-poly(methyl acrylate) (PAA-*b*-PMA) with, **1**, and without, **2**, a biotin chain terminus, were prepared for co-micellization, followed by intramicellar cross-linking. Nanostructures were prepared using 0%, 0.2%, 1%, 2%, 10%, 40%, and 100% biotinylated block copolymer. These ratios were selected to span from low numbers of biotin to full biotinylation.

The synthesis of block copolymer, **1**, having a biotin unit at the hydrophilic chain terminus, utilized a biotinylated initiator, **3**, for atom transfer radical polymerization (ATRP) (Scheme 2). Biotin, **4**, was first activated by 1,1'-carbonyldiimidazole, followed by coupling to a hydrophilic linker 2-(2'-aminoethoxy)-ethanol to form the biotinylated alcohol, **5**. Esterification of **5** with 2-bromo-2-methyl propionic acid, mediated by 4-(*N*,*N*-dimethylamino)pyridine (DMAP) and 1,3-dicyclohexylcarbodiimide (DCC), afforded **3** in 43% yield after purification by flash chromatography. The composite of ^{1}H NMR spectra (Figure 1) indicates the formation of **3**, wherein the methylene resonance for the protons labeled as H_j of **5** shifted downfield to 4.2 ppm, overlapping with the protons H_e and H_f on the biotin unit, upon formation of **3**. The appearance of the singlet resonating at 1.9 ppm confirmed the presence of the isobutyryl methyl groups of **3**. The urea protons on biotin were not observed, due to the rapid proton exchange with the solvent, CD_3OD.

Preparation of the block copolymers, **9** and **10**, was accomplished by sequential ATRP of *tert*-butyl acrylate and methyl acrylate, initiated from **3** and **6**, respectively (Scheme

(102) Ma, Q.; Remsen, E. E.; Clark, C. G., Jr.; Kowalewski, T.; Wooley, K. L. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 5058-5063.
(103) Ma, Q.; Remsen, E. E.; Kowalewski, T.; Wooley, K. L. *J. Am. Chem. Soc.* **2001**, *123*, 4627-4628.
(104) Huang, H.; Kowalewski, T.; Remsen, E. E.; Gertzmann, R.; Wooley, K. L. *J. Am. Chem. Soc.* **1997**, *119*, 11653-11659.
(105) Tian, M.; Qin, A.; Ramireddy, C.; Webber, S. E.; Munk, P.; Tuzar, Z.; Procházka, K. *Langmuir* **1993**, *9*, 1741-1748.
(106) Qin, A.; Tian, M.; Ramireddy, C.; Webber, S. E.; Munk, P.; Tuzar, Z. *Macromolecules* **1994**, *27*, 120-126.
(107) Bütün, V.; Wang, X. S.; de Paz Báñez, M. V.; Robinson, K. L.; Billingham, N. C.; Armes, S. P.; Tuzar, Z. *Macromolecules* **2000**, *33*, 1-3.

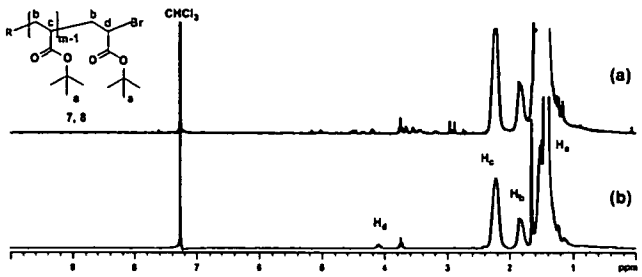


Figure 2. ^{1}H NMR spectra of (a) biotinylated P*t*BA, **7**, and (b) nonbiotinylated P*t*BA, **8**, in $CDCl_3$.

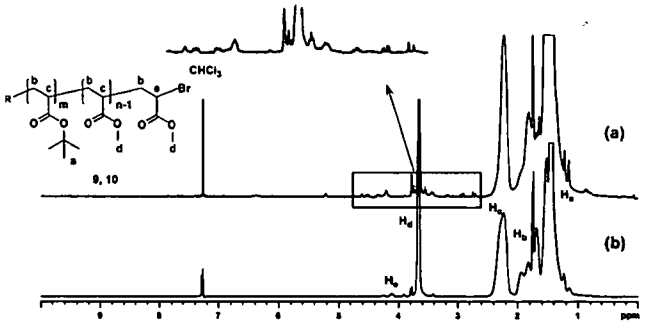


Figure 3. ^{1}H NMR of spectra (a) biotinylated P*t*BA-*b*-PMA, **9**, and (b) nonbiotinylated P*t*BA-*b*-PMA, **10**, in $CDCl_3$. A magnified view of proton resonances from the biotin unit is also shown.

3). Polymerization of *tert*-butyl acrylate initiated by **3**, using CuBr/*N*,*N*,*N*′,*N*″,*N*″-pentamethyldiethylenetriamine (PMDETA) as the catalyst/ligand system, was allowed to proceed at 55 °C to 76% conversion to give a 56% yield of biotinylated poly(*tert*-butyl acrylate) (P*t*BA) homopolymer, **7**. A small amount of *N*,*N*-dimethylformamide was added to solvate **3** and provide for a homogeneous polymerization mixture. Growth of the methyl acrylate chain segment from **7** was performed in bulk, in the presence of CuBr/PMDETA at 50 °C, and was allowed to proceed to <10% conversion, giving biotinylated poly(*tert*-butyl acrylate)-*b*-poly(methyl acrylate) (P*t*BA-*b*-PMA) diblock copolymer, **9**, in 41% yield. The synthesis of the nonbiotinylated P*t*BA homopolymer, **8**, and the corresponding P*t*BA-*b*-PMA diblock copolymer, **10**, followed a similar route, using ethyl 2-bromopropionate, **6**, as the ATRP initiator. The presence of the biotin functional group at the chain terminus was confirmed by ^{1}H NMR spectroscopy for the biotinylated homopolymer, **7**, and the biotinylated diblock copolymer, **9**, which are shown in Figures 2 and 3, in comparison to their nonbiotinylated analogues **8** and **10**, respectively. In Figure 3, a magnified view of a region of the ^{1}H NMR spectrum for the biotinylated diblock copolymer is provided to illustrate the resonances for H_c, H_d, H_e, and H_f of the biotin unit. The molecular weights and molecular weight distributions of polymers, **7**−**10**, were determined by size exclusion chromatography (SEC), equipped with multiangle laser light scattering and refractive index detection. The molecular weights were also determined via ^{1}H NMR end group analysis. The results are summarized in Table 1.

The *tert*-butyl ester groups on **9** and **10** were cleaved selectively by reaction with trifluoroacetic acid (TFA) in dichloromethane for ca. 14 h at room temperature. After removal of the solvent and excess TFA, the residue was dissolved in THF, and the amphiphilic block copolymers were purified by dialysis against deionized water (cellulose membrane dialysis tubing, MWCO 6000−8000 Da). Isolation of the amphiphilic

Scheme 3

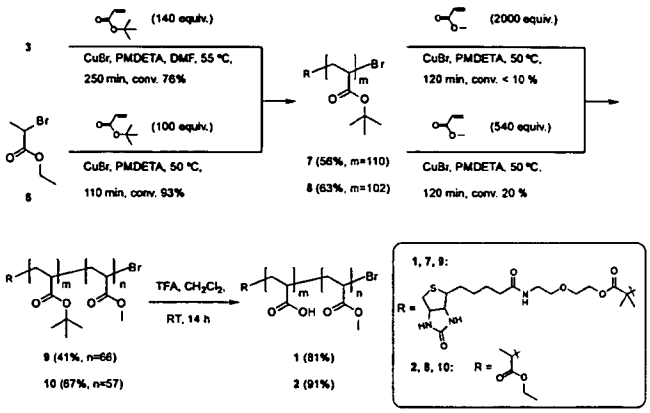


Table 1. Molecular Weights and Molecular Weight Distributions for Biotinylated and Nonbiotinylated P*t*BA$_m$ Homopolymers and P*t*BA$_m$-*b*-PMA$_n$ Diblock Copolymers by ^{1}H NMR and SEC

polymer	m^a	n^a	M_n (^{1}H NMR)^b	M_n (SEC)	M_w/M_n
7	110	0	17 200	14 400	1.27
8	102	0	12 500	13 200	1.22
9	110	66	25 200	20 100	1.01
10	102	57	15 400	18 100	1.09

^{a} Number of *tert*-butyl acrylate repeating units and methyl acrylate repeating units based on SEC characterization. ^{b} Molecular weights of biotinylated polymers were determined by comparison of the integration area of the average of the protons on the biotinylated initiator at the chain end to that of the resonance of the methine groups on the polymer backbone between 2 and 2.4 ppm, in the ^{1}H NMR spectra. Molecular weights of nonbiotinylated polymers were determined by comparison of the integration area of the average of the methylene proton resonance of the ethyl ester groups and the methine group at the chain ends to that of the resonance of the methine groups on the polymer backbone between 2 and 2.4 ppm, in the ^{1}H NMR spectra.

block copolymers, **1** and **2**, was then accomplished by lyophilization (Scheme 3). The selective cleavage of the *tert*-butyl groups was demonstrated by the disappearance of the *tert*-butyl proton resonance at 1.42 ppm in the ^{1}H NMR spectra and by the disappearance of the *tert*-butyl group stretching bands at 1393 and 1367 cm^{-1} in the IR spectra. In addition, the broadening of the carbonyl stretching band and the absorption from 3500 to 2500 cm^{-1}, characteristic of carboxylic acids, indicated the formation of PAA from P*t*BA. Although a number of solvents and solvent mixtures were employed (e.g., DMSO, THF/D_2O), upon cleavage of the *tert*-butyl groups of **9** to form amphiphilic block copolymer, **1**, the resonances for the protons of the biotin unit were no longer visible by ^{1}H NMR spectroscopy. The lack of observation of the chain end unit is consistent with a solubility behavior for a polymer having very different chain segment compositions. The primary ester linkage between the biotin unit and the polymer is stable under the TFA reaction conditions, which was confirmed by model studies whereby the biotinylated initiator treated under the same conditions for the cleavage of biotinylated PAA-*b*-PMA, **9**, was found to undergo no cleavage, as observed by ^{1}H NMR spectroscopy. Furthermore, confirmation of the persistence of the biotin chain end unit was made through assays that identified its presence on the surface of the micelles and SCK nanostructures, as described below. Glass transition temperatures T_g of **1**, **2**, and **7**−**10** were measured by differential scanning calorimetry (DSC) (Table 2).

The micellization process followed a two-step procedure. Amphiphilic block copolymer PAA-*b*-PMA was dissolved in THF (a solvent for the PMA and PAA segments), followed by

Table 2. Glass Transition Temperatures for Biotinylated and Nonbiotinylated Homopolymers and Diblock Copolymers Characterized by DSC

polymer	T_g (°C)[a] (P*t*BA)	T_g (°C)[a] (PMA)	T_g (°C)[a] (PAA)
7	45		
8	43		
9	41	not observed	
10	43	16	
1		not observed	124
2		13	123

[a] Measurements were performed with a heating rate of 10 °C/min under N_2 flow. T_g was taken as the midpoint of the inflection tangent upon the third or subsequent heating scans.

Table 3. Mixed Micelle Formation and DLS Characterization for the Mixed Micelles

micelle	biotinylated block copolymer content[a] (mol %)	amount of 1 (mg)	amount of 2 (mg)	THF amount (mL)	H_2O amount (mL)	micelle concentration (mg/mL)	D_h (nm) (number-average)
11	0%	0	60.0	60	60	0.261	26 ± 5
12	1%	0.7	58.6	60	60	0.253	27 ± 2
13	2%	1.3	58.0	60	60	0.245	28 ± 1
14	10%	6.8	62.0	64	64	0.271	29 ± 3
15	40%	12.0	18.6	30	30	0.267	32 ± 4

[a] Biotinylated block copolymer content was calculated on the basis of the molar percentage of the biotinylated PAA-*b*-PMA block copolymer to the nonbiotinylated PAA-*b*-PMA block copolymer.

the gradual addition of deionized water (a nonsolvent for PMA) into the polymer THF solution. Normally, equal volumes of water and THF were added to obtain spherical micelles with good reproducibility. The micelle stability was further established upon dialysis of the mixed H_2O/THF solution against deionized water to remove the THF solvent. The calculated concentration of the micelle solution was determined by measuring the final volume of the micelle obtained together with the initial weight of the polymer precursors used. By controlling the initial molar ratio of the biotinylated PAA-*b*-PMA to the nonbiotinylated PAA-*b*-PMA, a series of micelles and mixed polymer micelles, **11**−**15**, were formed, with a theoretical biotinylated chain incorporation of 0%, 1%, 2%, 10%, and 40%, respectively. The hydrodynamic diameters (D_h) of the micelles, **11**−**15**, were determined by dynamic light scattering (DLS) (Table 3).

Intramicellar cross-linking of the polymer micelles was achieved by intermolecular bond formation between the carboxylic acid groups in the polymer micelle shell layers, by activation with 1-[3′-(dimethylamino)propyl]-3-ethylcarbodiimide methiodide and reaction with 2,2′-(ethylenedioxy)diethylamine. Each of the micelles was cross-linked at a calculated mean-cross-linking density of 60%, based on the stoichiometry of amine functional groups of the diamino cross-linker to the carboxylic acid groups from the PAA-*b*-PMA block copolymers. The SCKs, **16**−**20**, having varying degrees of biotinylated block copolymer content, were purified by dialysis against deionized water (cellulose membrane tubing, MWCO 6000−8000 Da) to remove the unreacted cross-linker and byproducts. The amide bond formation was confirmed by IR spectroscopy with the introduction of amide I and II bands at ca. 1640 and 1560 cm^{-1}.

DLS characterization of SCKs in aqueous solution provided intensity autocorrelation functions that were deconvoluted into intensity-average hydrodynamic diameter distributions using CONTIN. The reliability of the analysis was confirmed by the

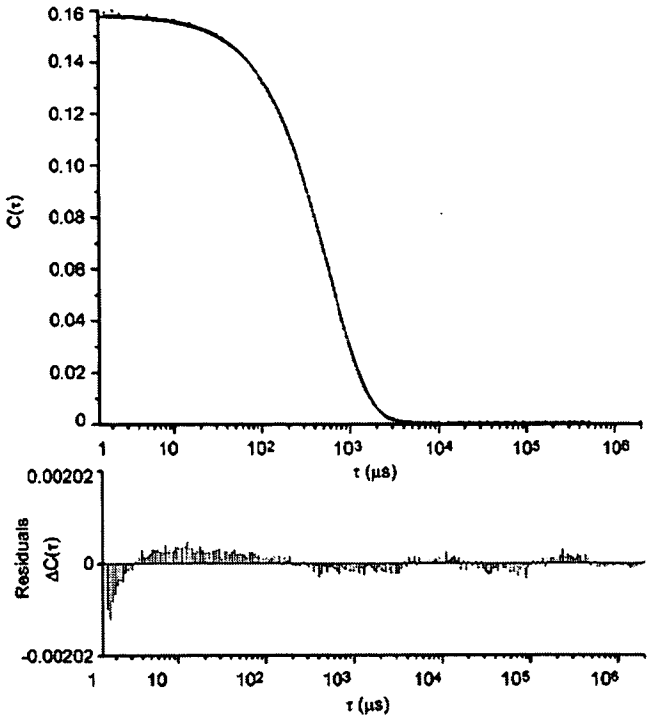


Figure 4. Intensity autocorrelation functions for the 2% biotinylated SCK, **18** (dots), and the CONTIN fit (line) at 30° scattering angle, and the residual plots for the corresponding CONTIN fit are also shown.

excellent agreement between CONTIN-computed autocorrelation functions and experimental autocorrelation functions as exemplified by the representative results shown for the 2% biotinylated SCK, **18**, in Figure 4. Small residuals found for the CONTIN analyses and depicted graphically in Figure 4 produced RMS errors on the order of 10^{-4} for all SCKs studied.

The intensity-average diameter distribution provided by CONTIN was geometrically transformed, assuming a spherical shape for the SCKs, into a volume-average diameter distribution. This was accomplished by dividing the volume fraction of nanoparticles, $V(D)$, with diameter, D, in the intensity-average diameter distribution by the product of the angular Mie scattering coefficient, $P(\theta)$, for the nanoparticle and the cube of its diameter, D^3. Under the assumption that all particles in the volume-average distribution have the same density, the volume-average distribution is equivalent to the weight-average diameter distribution.

The resulting volume-average diameter distribution was transformed into a number-average diameter distribution by dividing $V(D)$ for volume-average diameter distribution by D^3. The use of the volume- and number-average hydrodynamic diameter distributions afforded mean volume and number hydrodynamic diameters, $D_{h,volume}$ and $D_{h,number}$, respectively. The ratio, $D_{h,volume}/D_{h,number}$, served as a measure of polydispersity for the DLS-derived hydrodynamic diameter distribution that was not skewed by the minute fraction of aggregates present in aqueous solutions of the SCKs. As shown in Table 4, $D_{h,volume}/D_{h,number}$ ranged from 1.11 to 1.24 across the set of SCKs, indicating that the distributions of hydrodynamic diameter were narrow as shown in Figure 5. Measurements of hydrodynamic diameter distributions conducted at two additional angles, 30° and 125°, confirmed the finding of narrow hydrodynamic diameter distributions for all SCKs studied. In the case of micelles and SCKs prepared from 100% biotinylated PAA-*b*-

Table 4. DLS Characterization for the Biotinylated and the Nonbiotinylated SCKs

SCK[a]	biotinylated block copolymer content (mol %)	SCK concentration (mg/mL)	D_h (nm) (intensity-average)	D_h (nm) (volume-average)	D_h (nm) (number-average)	$D_{h,volume}/D_{h,number}$
16	0%	0.260	73 ± 2	31 ± 1	25 ± 2	1.24
17	1%	0.249	33 ± 3	29 ± 2	25 ± 1	1.16
18	2%	0.244	36 ± 7	31 ± 1	28 ± 1	1.11
19	10%	0.263	46 ± 1	30 ± 2	25 ± 2	1.20
20	40%	0.253	38 ± 1	30 ± 1	26 ± 1	1.15

[a] SCKs were prepared with 60% average cross-link density, as based on the ratio of amine functional groups from the diamino cross-linker to the carboxylic acid groups from the PAA-*b*-PMA block copolymer.

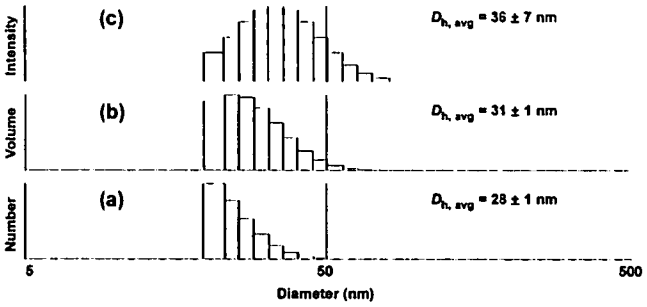


Figure 5. DLS characterization of 2% biotinylated SCK, **18**, in aqueous solution at 20 °C. Histograms are averaged by intensity, volume, and number, respectively (CONTIN fit).

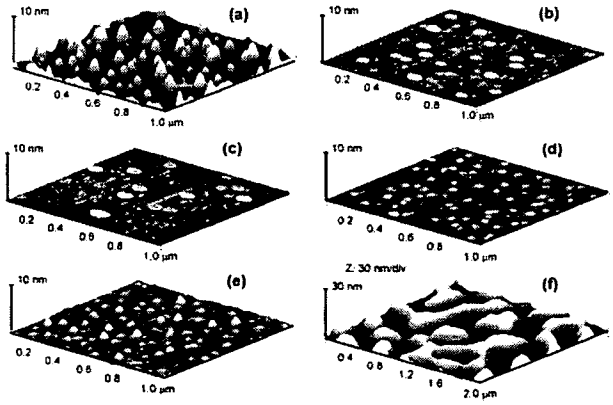


Figure 6. Tapping-mode AFM images of SCKs: (a) **16**, (b) **17**, (c) **18**, (d) **19**, (e) **20**, and (f) 100% biotinylated SCK. Samples were prepared by drop deposition onto freshly cleaved mica and allowed to dry in air.

PMA copolymers, species characterized by sizes greater than 100 nm and by nonspherical, irregular shapes were found by DLS and AFM measurements, respectively. Thus, low percentages of biotinylated block copolymer contents were chosen, ranging from 0% to 40% as mentioned above, for the study of their interactions with avidin.

The dimensions of the SCKs were characterized by tapping-mode atomic force microscopy (AFM) and transmission electron microscopy (TEM). The diameter values obtained by AFM (Figure 6) and TEM (Figure 7) were substantially larger than were the heights measured by AFM. These discrepancies are the result of the low T_g of the methyl acrylate core domain allowing for the particles to deform upon adsorption onto the solid substrates employed for AFM and TEM imaging.[74,75] In addition, the larger diameter values obtained from AFM measurements in comparison to those from TEM indicate greater deformation of the particles on the hydrophilic mica surface, the substrate for AFM characterization, as opposed to the hydrophobic carbon surface, the substrate for TEM characterization.[75] The characterization data for the SCK samples are summarized in Table 5.

Table 5. Size Characterization Data for the SCKs

SCK	biotinylated block copolymer content (mol %)	D_h[a] (nm) (DLS)	H_{av}[b] (nm) (AFM)	D_{av}[b] (nm) (AFM)	D_{av}[c] (nm) (TEM)
16	0%	25 ± 2	2.6 ± 0.6	68 ± 8	30 ± 5
17	1%	25 ± 1	1.2 ± 0.5	102 ± 8	27 ± 5
18	2%	28 ± 1	1.3 ± 0.4	84 ± 11	24 ± 4
19	10%	25 ± 2	0.5 ± 0.2	65 ± 12	36 ± 7
20	40%	26 ± 1	1.0 ± 0.4	71 ± 8	26 ± 4

[a] Number-average hydrodynamic diameters of SCKs in aqueous solution were characterized by dynamic light scattering. [b] Average heights and average diameters of SCKs were measured by tapping-mode AFM, averaged from the diameters of ca. 150 particles. [c] Average diameters of SCKs were measured by TEM, averaged from the diameters of ca. 150 particles.

Avidin/HABA Binding Assay. The bioavailability of biotin presented from the SCKs to its protein receptor was evaluated by a competitive binding assay (avidin/HABA) and fluorescence correlation spectroscopy (FCS) studies. The amount of surface-available biotin on each functionalized SCK sample was quantified by the avidin/HABA assay.[108–111]

The results of these analyses are shown in the overlaid UV–vis spectra (Figure 8a). Upon addition of SCK nanoparticles with different biotinylated block copolymer content, the change of absorbance at 500 nm increased with an increase in biotinylated block copolymer content, suggesting the avidin/HABA complex is displaced by biotin presenting on the SCK surface. The amount of biotin in each of the biotinylated SCK sample solutions was calculated, and the data are summarized in Table 6. The amount of available biotin for each of the biotinylated SCK nanoparticles corresponded to nanomoles of biotin per milliliter of SCK sample, which increased with an increase in biotinylated block copolymer content, controlled by the initial stoichiometry of **1**:2 during the process of mixed micelle formation.

To compare the relative amount of surface-available biotin among SCK samples, the relative amounts of biotin on the SCK surfaces were calculated by correction for concentration differences and normalization with the amount of surface-available biotin using the 1% biotinylated SCK, **17**, as the normalization standard. The normalized amount of biotin, available for interaction with avidin, for each sample is plotted against the percentage of biotinylated block copolymer content in Figure 8c. The relative numbers of biotin units exposed on the SCK surface and available for binding with avidin increased as the theoretical numbers increased.

To estimate the functional group availability obtained via the mixed micelle methodology, the amount of measured biotin based on the avidin/HABA assay was compared against the

(108) Green, N. M. *Biochem. J.* **1965**, *94*, 23c–24c.
(109) Green, N. M. *Methods Enzymol.* **1970**, *18*, 418–424.
(110) Savage, M. D. *A Laboratory Guide to Biotin-Labeling in Biomolecule Analysis*; Meier, T., Fahrenholz, F., Eds.; Birkhäuser Verlag: Basel, Boston, Berlin, 1996; pp 1–28.
(111) HABA is a dye that binds to avidin in the same binding pocket used to bind biotin. HABA has a maximum UV absorbance at 350 nm, and once an avidin/HABA complex has formed, the maximum UV absorbance shifts to 500 nm. As compared with the strong affinity for biotin exhibited by avidin ($K_d = 10^{-15}$ M), avidin's affinity for HABA is much weaker ($K_d = 10^{-6}$ M). Thus, when biotin is added to a solution of avidin/HABA complex, HABA is displaced quantitatively by biotin, and this displacement can be quantitatively monitored by the decrease in UV absorbance at 500 nm.

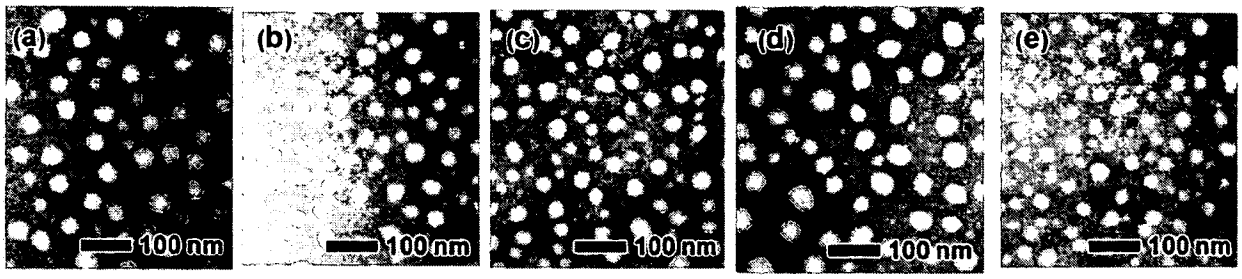


Figure 7. TEM images of SCKs: (a) **16**, (b) **17**, (c) **18**, (d) **19**, and (e) **20**. Samples were stained with phosphotungstic acid and drop deposited onto a carbon-coated copper grid.

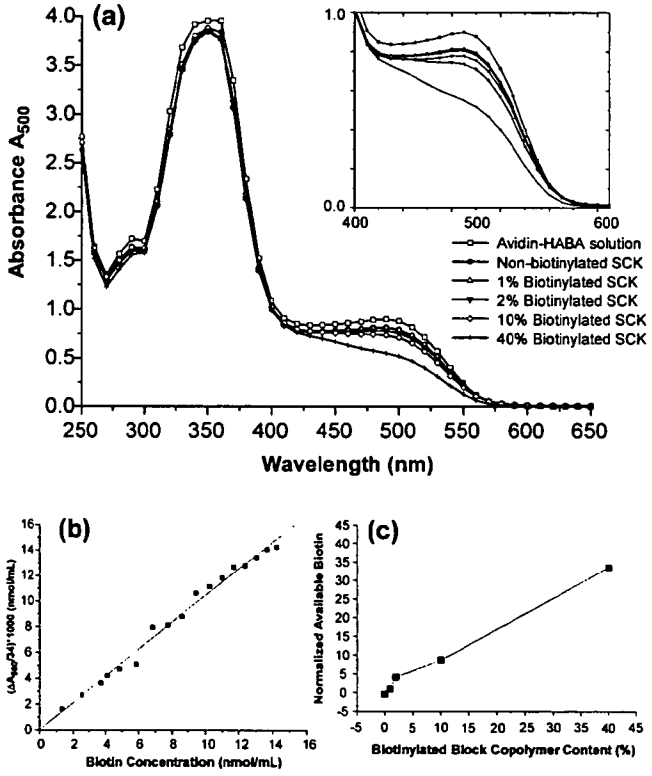


Figure 8. (a) UV–vis spectra of the avidin–HABA complex and the avidin–HABA complex added with **16**–**20**, respectively, in 50 mM PBS buffer, 50 mM NaCl, pH 7.1. The zoomed region at 500 nm is shown in the subset. (b) Calibration curve for avidin/HABA assay. (c) Normalized amount of surface-available biotin versus the biotinylated block copolymer content (**16**–**20** corresponding to 0%, 1%, 2%, 10%, and 40%, respectively).

theoretical values, which were calculated on the basis of the initial stoichiometry of **1** and **2** being used in the preparation of the mixed micelles (Table 6). The surface accessibilities of the biotin units for the micelles and SCKs were similar, as determined for the samples having 10% biotinylated block copolymer incorporation, **19**, **21**, and **22**.[112] It is uncertain whether the degree of shell cross-linking of the SCKs and rigidity of the particles play a role in the bioavailability of the surface-accessible groups. Within the experimental error, the percentage of biotin units available in comparison to those incorporated in the formulation remained consistently at 10–25%. The less than complete surface accessibility and bioavailability can be explained by several effects, although the magnitude of the contributions from each of these potential effects is unknown. The most likely cause of reduced biotin availability was trapping of biotinylated chain ends below the nanoparticle surface during micelle formation. Other potential sources of inhibition for biotin binding include SCK cross-linking density, surface rigidity, chain dynamics, and polydispersity.

Fluorescence Correlation Spectroscopy (FCS). The multivalent binding event expected for biotinylated-SCK:protein receptor interactions was also studied using FCS. The measurement of the translational diffusion coefficient for a fluorescent species within a small focal volume is provided by FCS.[113–119] Because the diffusion of spherical molecules is inversely proportional to the cube root of the molecular weight, any association or binding interaction that leads to the increase of molecular weight will result in a decrease of the diffusion coefficient, which can be identified and quantified by the correlation time from an autocorrelation analysis of fluorescence intensity fluctuations.

The FCS instrument was home-built,[120] based on the principle of a solid immersion microscope,[121–124] and the schematic drawing of the instrumentation setup was shown in Figure 9a. Alexa Fluor 488 labeled avidin was analyzed by FCS, and a hydrodynamic diameter of 9.2 nm was found. This diameter, as expected, is small as compared with values obtained for nonfluorescence SCK nanoparticles using DLS (D_h ca. 30 nm). As a result, SCK:protein binding due to biotin–avidin recognition was expected to decrease the diffusion coefficient of the fluorescently labeled avidin. In addition, as more avidin was bound to SCK nanoparticles, a larger fraction of the slower diffusing SCK:protein complex was anticipated.

FCS measurements of Alexa Fluor 488 labeled avidin and SCKs, **16**–**20**, with different biotinylated block copolymer contents were performed in 10 mM of HEPES buffer with 1 mM EDTA, 1 M NaCl, and at pH 7.1. A high salt concentration was used to eliminate the nonspecific binding interactions.[125]

(112) Sample **21** is a 10% biotinylated micelle, prepared by mixing **1** with nonbiotinylated PAA_{93}-*b*-PMA_{76}, M_n^{SEC} = 18 600 g/mol, M_w/M_n = 1.12, which is slightly different from the nonbiotinylated block copolymer, **2**, used to prepare **19**, and sample **22** is the corresponding SCK prepared from **21**, with 60% cross-linking density.

(113) Elson, E. L.; Magde, D. *Biopolymers* **1974**, *13*, 1–27.
(114) Magde, D.; Elson, E. L.; Webb, W. W. *Biopolymers* **1974**, *13*, 29–61.
(115) Schwille, P.; Oehlenschläger, F.; Walter, N. G. *Biochemistry* **1996**, *35*, 10182–10193.
(116) Wohland, T.; Friedrich, K.; Hovius, R.; Vogel, H. *Biochemistry* **1999**, *38*, 8671–8681.
(117) Schüler, J.; Frank, J.; Trier, U.; Schäfer-Korting, M.; Saenger, W. *Biochemistry* **1999**, *38*, 8402–8408.
(118) Medina, M. A.; Schwille, P. *BioEssays* **2002**, *24*, 758–764.
(119) Haustein, E.; Schwille, P. *Methods* **2003**, *29*, 153–166.
(120) Clark, C. G., Jr.; Remsen, E. E.; Wooley, K. L., manuscript in preparation.
(121) Mansfield, S. M.; Kino, G. S. *Appl. Phys. Lett.* **1990**, *57*, 2615–2616.
(122) Ghislain, L. P.; Elings, V. B. *Appl. Phys. Lett.* **1998**, *72*, 2779–2781.
(123) Ghislain, L. P.; Elings, V. B.; Crozier, K. B.; Manalis, S. R.; Minne, S. C.; Wilder, K.; Kino, G. S.; Quate, C. F. *Appl. Phys. Lett.* **1999**, *74*, 501–503.
(124) Koyama, K.; Yoshita, M.; Baba, M.; Suemoto, T.; Akiyama, H. *Appl. Phys. Lett.* **1999**, *75*, 1667–1669.
(125) Swamy, M. J.; Marsh, D. *Biochemistry* **2001**, *40*, 14869–14877.

Table 6. Results of the Avidin/HABA Assay for the Micelle and SCK Samples

sample	biotinylated block copolymer content (mol %)	sample concentration[a] (mg/mL)	theoretical value of available biotin[b] (nmol/g)	$(\Delta A_{500}/34) \times 1000$[c] (nmol/mL)	available biotin per milliliter of sample solution[d] (nmol/mL)	available biotin per gram of polymer[e] (nmol/g)	fraction of surface-available biotin[f]
16	0%	2.53	0	0	0	0	n/a
17	1%	2.59	900	0.3	0.3	99	11%
18	2%	1.39	1680	0.7	0.6	410	25%
19	10%	2.93	7560	2.7	2.5	850	11%
21	10%	2.63	7520	4.3	4.1	1600	22%
22	10%	2.51	7520	3.7	3.4	1400	19%
20	40%	2.45	30 000	8.2	8.1	3300	11%
15	40%	1.38	30 000	5.0	4.8	3500	12%

[a] The micelle and SCK samples were concentrated using a stirred ultrafiltration cell equipped with an ultrafiltration membrane filter disk (NMWL 100 000 Da). [b] Theoretical values of the surface-available biotins based on the initial molar ratios of the biotinylated PAA-*b*-PMA to the nonbiotinylated PAA-*b*-PMA during the preparation; the data are represented in units of nanomoles of biotin per gram of polymer precursor mixture. [c] $(\Delta A_{500}/34) \times 1000$ was used to calculate the amount of biotin in the sample solution being analyzed in nmol/mL. [d] Surface-available biotin in the SCK or micelle solution by comparison of the absorbance change with that of the calibration curve. [e] Surface-available biotin per gram of polymer precursor mixture based on the calculated concentration of the sample solutions used in each assay. [f] Fraction of the surface-available biotin is the molar percentage of available biotin to the theoretical value.

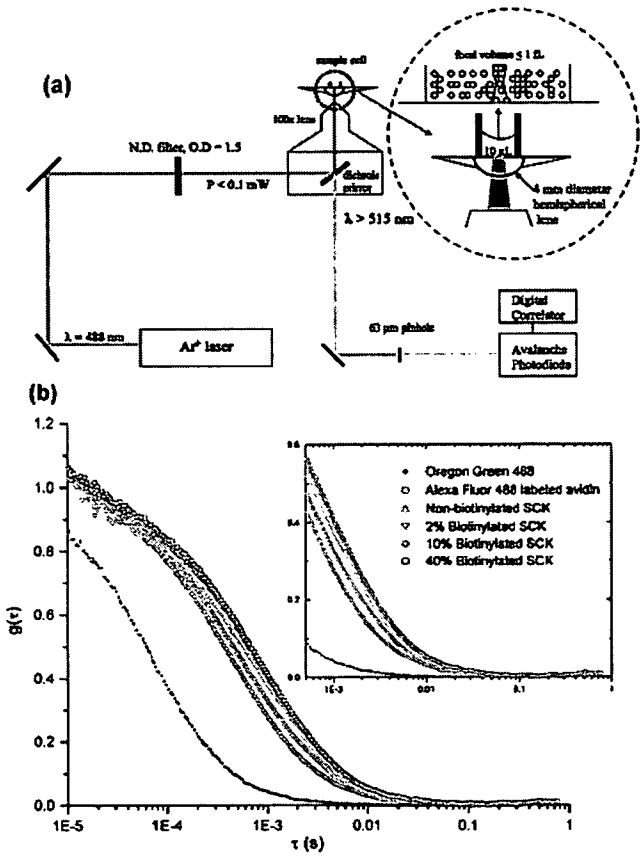


Figure 9. (a) Schematic drawing of FCS instrumentation setup; (b) normalized autocorrelation function of Oregon Green 488, Alexa Fluor 488 conjugate, and mixture of Alexa Fluor 488 conjugate with **16**–**20**, in 10 mM HEPES buffer with 1 mM EDTA and 1 M NaCl, pH 7.1, respectively. A zoomed region of the autocorrelation function is shown in the subset.

The same concentration of the labeled avidin used to characterize the diffusion coefficient for a solution of pure labeled protein was employed for each SCK:protein solution mixture. Normalized fluorescence intensity autocorrelation functions for SCK:protein mixtures, the pure labeled protein, and an Oregon Green 488 calibration standard are shown in Figure 9b.

The correlation time τ_d for each measurement was calculated by fitting the autocorrelation function. To calculate the diffusion coefficient of the samples, Oregon Green 488 was used to characterize the focal volume. The correlation time was converted to diffusion coefficient by reference to the τ_d of

Table 7. Data Summary for the FCS Measurement

	N	$\tau_d \times 10^{-5}$ (s)	$D_T \times 10^{-11}$ ($m^2\ s^{-1}$)	D_h[c] (nm)	D_h[d] (nm)	X_{bound}[e]
OG[a]	8.2	6.4 ± 0.7	27 ± 3	1.5 ± 0.2		
avidin[a]	0.67	41.7 ± 0.5	4.1 ± 0.6	9.2 ± 1.4		
16	0.72	50.5 ± 0.6	3.4 ± 0.5	11.2 ± 1.7	31 ± 1	0.14
18	0.76	62.1 ± 0.9	2.8 ± 0.4	13.8 ± 2.2	31 ± 1	0.32
19	0.87	65.1 ± 1.0	2.6 ± 0.4	14.4 ± 2.2	30 ± 2	0.42
20	0.79	73.0 ± 1.0	2.4 ± 0.4	16.2 ± 2.6	30 ± 1	0.41
20[b]	17	78.4 ± 0.6	2.2 ± 0.3	17.4 ± 2.7	30 ± 1	0.47

[a] OG is Oregon Green 488; avidin is Alexa Fluor 488 labeled avidin. [b] The same 40% biotinylated SCK sample, with a 10-fold increase of the avidin concentration. [c] Hydrodynamic diameters of the fluorescent species calculated from correlation time τ_d. [d] Volume-average hydrodynamic diameters of SCKs from DLS measurements, which were used to calculate τ_{bound} for the fitting into the two-component model, eq 5 in the Supporting Information. [e] Fraction of avidin–SCK complex, calculated by fitting with eq 5 (Supporting Information).

Oregon Green 488, whose diffusion coefficient was determined via calibration with Rhodamine 6G having a known diffusion coefficient of 2.80×10^{-10} $m^2\ s^{-1}$ (at 22 °C).[126] Correlation times and diffusion coefficients are summarized in Table 7. The correlation time τ_d increased as the biotinylated block copolymer content in each SCK sample increased, indicating more labeled avidin was bound to SCK per unit concentration of avidin in the solution, which additionally suggested that more biotin was available to its protein receptors. The averaged hydrodynamic diameters of the fluorescent species in each solution were calculated using eqs 3 and 4 (Supporting Information), by employing Oregon Green 488 as a calibrant for the calculation of D_T. The fluorescent species in solution were also analyzed as either free labeled avidin or labeled avidin-bound SCKs using a two-component model, eq 5 (Supporting Information). The calculation of diffusion coefficients and the averaged hydrodynamic diameters of the fluorescent species using Oregon Green 488 as calibrant have an experimental error up to 16% in each determination, which mainly arise from the determination of the correlation time of Oregon Green 488. However, the correlation time determination for each sample is independent of the calibrant, so the trend observed in the determination is valid. The calculations for the bound fraction of the fluorescent species using the two-component model are also not affected.

(126) Rigler, R.; Mets, Ü.; Widengren, J.; Kask, P. *Eur. Biophys. J.* **1993**, *22*, 169–175.

In agreement with the avidin/HABA assay, these FCS measurements found that the amount of available biotin at the surface of the biotinylated SCK nanoparticles increased with increasing biotin-terminated block copolymer incorporation, as evidenced by the increasing correlation times, and the fraction of biotin that underwent binding with avidin was ca. constant. To confirm there was no significant amount of available biotin that remained unbound, the 40% biotinylated SCK sample was also analyzed with an avidin concentration increased by 10-fold. Under conditions of excess avidin, the lack of a significant change of the hydrodynamic diameter of the avidin−SCK complex and the presence of free avidin indicated a saturation of the biotinylated SCKs with labeled avidin.

Conclusions

A novel biotinylated initiator was synthesized and utilized to initiate, via ATRP, homo- and diblock copolymer bearing a single biotin moiety at the chain terminus. The chain terminal biotinylated amphiphilic diblock copolymer PAA-*b*-PMA was mixed in solution with its nonbiotinylated analogue to form mixed micelles. The mixed micelle approach offers control over the functional group incorporation within the nanoparticles by varying the stoichiometric ratio of the functionalized to non-functionalized micelle precursors. Intramicellar cross-linking within the PAA shell layer converted the supramolecular assemblies to robust SCK nanoparticles presenting bioactive biotin.

The surface- and bioavailability of the biotinylated SCK nanoparticles, comprised of different percentages of biotinylated block copolymer, were evaluated using an avidin/HABA competitive binding assay and FCS. Data from these studies support the trend that greater numbers of biotin were available to bind with the protein receptors with an increase in the biotinylated block copolymer incorporation. It is important to note that the shell cross-linking of the nanostructure presenting the biotin did not diminish the bioavailability for binding with the biological macromolecules. The avidin/HABA assay quantified the amount of available biotin to be less than 25% of the theoretical value, likely due to the loss of functional group availability in the process of micellization and shell cross-linking. However, kinetic studies that remain in progress are revealing that the binding interactions are significantly more complex than is considered here. These preliminary studies indicate that there are kinetic differences and differences in the ultimate level of available biotin units observed between the samples; the rates of binding increase and the percentage of available biotin units decrease with increasing biotinylation. FCS results indicated the same trend for the binding capacity of 10% and 40% biotinylated SCKs. With the enhanced sensitivity of fluorescence cross-correlation spectroscopy,[118] the binding isotherm of the biotinylated SCKs of different biotin surface coverage might be determined accurately, potentially advancing the understanding of the thermodynamics and kinetics of this multivalent model system. Geometric considerations, involving hard sphere surface contacts, indicate that approximately 60 avidins[127] can be accommodated through binding upon the surface of an SCK having a hydrodynamic diameter of 30 nm

(127) Using the angle (27.15°) between by two adjacent circles of radius 4.6 on the surface of a circle radius 15, the maximum number of smaller spheres that can be packed on the surface of the larger sphere is 60. Sloane, N. J. A.; Hardin, R. H.; Smith, W. D., and others. Spherical Codes. http://www.research.att.com/~njas/packings/index.html (accessed October 2003).

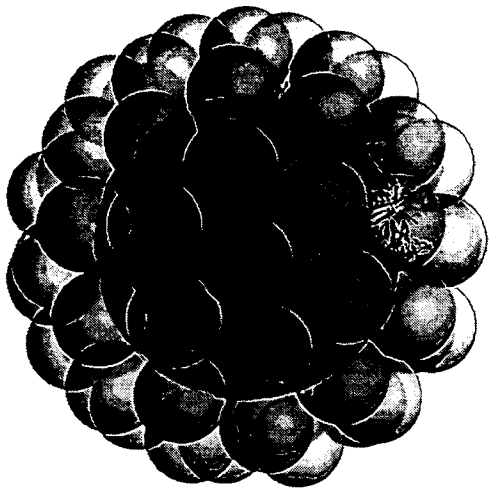


Figure 10. Schematic illustration of 60 avidins (with a diameter of 9.2 nm) packing at the surface of SCK (with a diameter of 30 nm) based on "hard sphere" surface contacts.

(Figure 10). Accurate values of the SCK aggregation numbers are yet to be determined, but they can be estimated to be between 400 and 800 when comprised from the diblock copolymers of ca. 13 000 Da, which suggests that there will be significant steric effects that will prevent the binding of avidins to all of the biotin units present.

These early findings are instructive, indicating that binding of biological macromolecules to the SCKs functionalized with bioactive moieties is a complex process that can be controlled, but which also requires substantial further investigation to be understood. These initial results demonstrate a mechanism for the preparation of well-defined nanostructured materials that serve as a model for the study of molecular ligands, immobilized on a nanoscopic surface, and recognized by their protein receptors, a pervasive phenomenon in biological systems. Further studies are in progress, utilizing the control in the preparation of biotinylated SCK nanoparticles, and in conjunction with the compositional versatility and structural stability of SCK nanoparticles as robust nanoscopic building blocks to form supramolecular assemblies, namely fabrication of 2-D and 3-D nanostructured materials with biotinylated SCKs employing biotin−avidin recognition.

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Supporting Information Available: Full experimental details and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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DESCRIPTION

METHODS FOR FABRICATING ISOLATED MICRO- AND NANO-STRUCTURES USING SOFT OR IMPRINT LITHOGRAPHY

TECHNICAL FIELD

Methods for preparing a patterned structure and methods for forming a pattern on a substrate using soft or imprint lithography. More particularly, methods for preparing a patterned micro or nano-structure and methods for forming a micro- or nano-scale pattern on a substrate using soft or imprint lithography.

ABBREVIATIONS

°C	=	degrees Celsius
cm	=	centimeter
DBTDA	=	dibutyltin diacetate
DMA	=	dimethylacrylate
DMPA	=	2,2-dimethoxy-2-phenylacetophenone
EIM	=	2-isocyanatoethyl methacrylate
FEP	=	fluorinated ethylene propylene
Freon 113	=	1,1,2-trichlorotrifluoroethane
g	=	grams
h	=	hours
Hz	=	hertz
IL	=	imprint lithography
kg	=	kilograms
kHz	=	kilohertz
kPa	=	kilopascal
MCP	=	microcontact printing
MHz	=	megahertz

mL	=	milliliters
mm	=	millimeters
mmol	=	millimoles
mN	=	milli-Newton
m.p.	=	melting point
mW	=	milliwatts
NCM	=	nano-contact molding
NIL	=	nanoimprint lithography
nm	=	nanometers
PDMS	=	polydimethylsiloxane
PFPE	=	perfluoropolyether
PP	=	polypropylene
psi	=	pounds per square inch
PVDF	=	poly(vinylidene fluoride)
PTFE	=	polytetrafluoroethylene
SAMIM	=	solvent-assisted micro-molding
SEM	=	scanning electron micrograph
S-FIL	=	"step and flash" imprint lithography
Si	=	silicon
TMPTA	=	trimethylopropane
µm	=	micrometers
UV	=	ultraviolet
W	=	watts
ZDOL	=	poly(tetrafluoroethylene oxide-*co*-difluoromethylene oxide)α,ω diol

BACKGROUND

The availability of viable nanofabrication processes is a key factor to realizing the potential of nanotechnologies. In particular, the availability of viable nanofabrication processes is important to the fields of photonics, electronic, and proteomics. Traditional imprint lithographic (IL) techniques are an alternative to photolithography for manufacturing integrated circuits, micro- and nano-fluidic devices, and other devices with micron sized features. There

is a need in the art, however, for new materials to advance IL techniques. See Xia, Y., et al., *Angew. Chem. Int. Ed.*, **1998**, *37*, 550-575; Xia, Y., et al., *Chem. Rev.*, **1999**, *99*, 1823-1848; Resnick, D. J., et al., *Semiconductor International*, **2002**, June, 71-78; Choi, K. M., et al., *J. Am. Chem. Soc.*, **2003**, *125*, 4060-4061; McClelland, G. M., et al., *Appl. Phys. Lett.*, **2002**, *81*, 1483; Chou, S. Y., et al., *J. Vac. Sci. Technol. B*, **1996**, *14*, 4129; Otto, M., et al., *Microelectron. Eng.*, **2001**, *57*, 361; and Bailey, T., et al., *J. Vac. Sci. Technol., B*, **2000**, *18*, 3571.

Imprint lithography comprises at least two areas: (1) so-called soft lithographic techniques, see Xia, Y., et al., *Angew. Chem. Int. Ed.*, **1998**, *37*, 550-575, such as solvent-assisted micro-molding (SAMIM); micro-molding in capillaries (MIMIC); and microcontact printing (MCP); and (2) rigid imprint techniques, such as nano-contact molding (NCM), see McClelland, G. M., et al., *Appl. Phys. Lett.*, **2002**, *81*, 1483; Otto, M., et al., *Microelectron. Eng.*, **2001**, *57*, 361; "step and flash" imprint lithographic (S-FIL), see Bailey, T., et al., *J. Vac. Sci. Technol., B*, **2000**, *18*, 3571; and nanoimprint lithography (NIL), see Chou, S. Y., et al., *J. Vac. Sci. Technol. B*, **1996**, *14*, 4129.

Polydimethylsiloxane (PDMS) based networks have been the material of choice for much of the work in soft lithography. See Quake, S. R., et al., *Science*, **2000**, *290*, 1536. The use of soft, elastomeric materials, such as PDMS, offers several advantages for lithographic techniques. For example, PDMS is highly transparent to ultraviolet (UV) radiation and has a very low Young's modulus (about 750 kPa), which gives it the flexibility required for conformal contact, even over surface irregularities without the potential for cracking. In contrast, cracking can occur with molds made from brittle, high-modulus materials, such as etched silicon and glass. See Bietsch, A., et al., *J. Appl. Phys.*, **2000**, *88*, 4310-4318. Further, flexibility in molds facilitates easy release of the mold from masters and replicates without cracking and allows the mold to endure multiple imprinting steps without damaging fragile features.

Although PDMS offers some advantages in soft lithography applications, a number of properties inherent to PDMS severely limit its

capabilities in soft lithography. First, PDMS-based elastomers swell when exposed to most oil-soluble organic compounds. See Lee, J. N., et al., *Anal. Chem.*, **2003**, *75*, 6544-6554. Although this property is favorable in MCP applications because it allows the mold to adsorb organic inks, see Xia, Y., et al., *Angew. Chem. Int. Ed.*, **1998**, *37*, 550-575, resistance to swelling is critically important in nearly all other soft lithographic techniques, especially for SAMIM and MIMIC, and for IL techniques in which a mold is brought into contact with a small amount of curable organic monomer or resin. Otherwise, the fidelity of the features on the mold is lost and an unsolvable adhesion problem ensues due to infiltration of the curable liquid into the mold. Such problems commonly occur with PDMS-based molds because most organic liquids swell PDMS. Organic liquids, however, are the materials most desirable to mold.

Secondly, the surface energy of PDMS (~25 mN/m) is not low enough for soft lithography procedures that require high fidelity. For this reason, the patterned surface of PDMS-based molds is often fluorinated using a plasma treatment followed by vapor deposition of a fluoroalkyl trichlorosilane. See Xia, Y., et al., *Angew. Chem. Int. Ed.*, **1998**, *37*, 550-575.

Third, the most commonly-used commercially available form of the material used in PDMS molds, e.g., Sylgard 184® (Dow Corning Corporation, Midland, Michigan, United States of America) has a modulus that is too low (~1.5 MPa) for many applications. The low modulus of these commonly used PDMS materials results in sagging and bending of features and, as such, is not well suited for processes that require precise pattern placement and alignment. Although researchers have attempted to address this last problem, see Odom, T. W., et al., *J. Am. Chem. Soc.*, **2002**, *124*, 12112-12113; Odom, T. W. et al., *Langmuir*, **2002**, *18*, 5314-5320; Schmid, H., et al., *Macromolecules*, **2000**, *33*, 3042-3049; Csucs, G., et al., *Langmuir*, **2003**, *19*, 6104-6109; Trimbach, D., et al., *Langmuir*, **2003**, *19*, 10957-10961, the materials chosen still exhibit poor solvent resistance and require fluorination steps to allow for mold release.

Rigid materials, such as quartz glass and silicon, also have been used in imprint lithography. See Xia, Y., et al., *Angew. Chem. Int. Ed.*, **1998**, *37*,

550-575; Resnick, D. J., et al., *Semiconductor International*, **2002**, June, 71-78; McClelland, G. M., et al., *Appl. Phys. Lett.*, **2002**, *81*, 1483; Chou, S. Y., et al., *J. Vac. Sci. Technol. B*, **1996**, *14*, 4129; Otto, M., et al., *Microelectron. Eng.*, **2001**, *57*, 361; and Bailey, T., et al., *J. Vac. Sci. Technol., B*, **2000**, *18*, 3571; Chou, S. Y., et al., *Science*, **1996**, *272*, 85-87; Von Werne, T. A., et al., *J. Am. Chem. Soc.*, **2003**, *125*, 3831-3838; Resnick, D. J., et al., *J. Vac. Sci. Technol. B*, **2003**, *21*, 2624-2631. Such materials are superior to PDMS in modulus and swelling resistance, but lack flexibility. Such lack of flexibility inhibits conformal contact with the substrate. Another drawback of rigid materials is the necessity to use a costly and difficult to fabricate hard mold, which is typically made by using conventional photolithography or e-beam lithography. See Chou, S. Y., et al., *J. Vac. Sci. Technol. B*, **1996**, *14*, 4129. More recently, the need to repeatedly use expensive quartz glass or silicon molds in NCM processes has been eliminated by using an acrylate-based mold generated from casting a photopolymerizable monomer mixture against a silicon master. See McClelland, G. M., et al., *Appl. Phys. Lett.*, **2002**, *81*, 1483, and Jung, G. Y., et al., *Nanoletters*, **2004**, ASAP.

Despite such advances, other disadvantages of fabricating molds from rigid materials include the necessity to use fluorination steps to the lower surface energy of the mold, see Resnick, D. J., et al., *Semiconductor International*, **2002**, June, 71-78, and the inherent problem of releasing a rigid mold from a rigid substrate without breaking or damaging the mold. See Resnick, D. J., et al., *Semiconductor International*, **2002**, June, 71-78; Bietsch, A., *J. Appl. Phys.*, **2000**, *88*, 4310-4318. Khang, D. Y., et al., *Langmuir*, **2004**, *20*, 2445-2448, have reported the use of rigid molds composed of thermoformed Teflon AF™ to address the surface energy problem. Fabrication of these molds, however, required high temperatures and pressures in a melt press, a process that could be damaging to the delicate features on a silicon wafer master. Further, these molds still exhibit the intrinsic drawbacks of other rigid materials outlined hereinabove. A clear and important problem of fabricating structures on semiconductor devices using molds or templates made from hard materials is the usual formation of

a "scum" layer that forms when a rigid template is brought into contact with a substrate. Even with elevated applied forces, it is very difficult to cleanly displace liquids during this process, which can result in the formation of a scum layer. Thus, there is a need in the art for a method of fabricating structures on a substrate, such as a semiconductor device, which does not result in the formation of a scum layer.

The fabrication of organic solvent resistant, microfluidic devices with features on the order of hundreds of microns from photocurable perfluoropolyether (PFPE) has been reported. See Rolland, J. P., et al., *J. Am. Chem. Soc.*, **2004**, *126*, 2322-2323. PFPE-based materials are liquids at room temperature and can be photochemically cross-linked to yield tough, durable elastomers. Further, PFPE-based materials are highly fluorinated and resist swelling by organic solvents, such as methylene chloride and acetonitrile, which are desirable for use in microchemistry platforms based on elastomeric microfluidic devices. There is a need in the art, however, to apply PFPE-based materials to the fabrication of nanoscale devices.

Further, there is a need in the art for improved methods for forming a pattern on a substrate, such as method employing a patterned mask. See U. S. Patent No. 4,735,890 to Nakane et al.; U. S. Patent No. 5,147,763 to Kamitakahara et al.; U.S. Patent No. 5,259,926 to Kuwabara et al.; and International PCT Publication No. WO 99/54786 to Jackson et al., each of which is incorporated herein by reference in their entirety.

There is also a need in the art for an improved method for forming isolated structures that can be considered "engineered" structures, including but not limited to particles, shapes, and parts. Using traditional IL methods, the scum layer that almost always forms between structures acts to connect or link structures together, thereby making it difficult, if not impossible to harvest isolated structures.

There also is a need in the art for an improved method for forming micro- and nanoscale charged particles, in particle polymer electrets. The term "polymer electrets" refers to dielectrics with stored charge, either on the surface or in the bulk, and dielectrics with oriented dipoles, frozen-in or ferroelectric. On the macro scale, such materials are used, for example, for

electronic packaging and charge electret devices, such as microphones and the like. See Kressman, R., et al., *Space-Charge Electrets*, Vol. 2, Laplacian Press, 1999; and Harrison, J. S., et al., *Piezoelectic Polymers*, NASA/CR-2001-211422, ICASE Report No. 2001-43. Poly(vinylidene fluoride) (PVDF) is one example of a polymer electret material. In addition to PVDF, charge electret materials, such as PP, Teflon-FEP, and PTFE, also are considered polymer electrets.

Further, there is a need in the art for improved methods for delivering therapeutic agents, such as drugs, non-viral gene vectors, DNA, RNA, RNAi, and viral particles, to a target. See *Biomedical Polymers*, Shalaby, S. W., ed., Harner/Gardner Publications, Inc., Cincinnati, Ohio, 1994; *Polymeric Biomaterials*, Dumitrin, S., ed., Marcel Dekkar, Inc., New York, New York, 1994; Park, K., et al., *Biodegradable Hydrogels for Drug Delivery*, Technomic Publishing Company, Inc., Lancaster, Pennsylvania, 1993; Gumargalieva, et al., *Biodegradation and Biodeterioration of Polymers: Kinetic Aspects*, Nova Science Publishers, Inc., Commack, New York, 1998; *Controlled Drug Delivery*, American Chemical Society Symposium Series 752, Park, K., and Mrsny, R. J., eds., Washington, D.C., 2000; *Cellular Drug Delivery: Principles and Practices*, Lu, D. R., and Oie, S., eds., Humana Press, Totowa, New Jersey, 2004; and *Bioreversible Carriers in Drug Design: Theory and Applications*, Roche, E. B., ed., Pergamon Press, New York, New York, 1987. For a description of representative therapeutic agents for use in such delivery methods, see U.S. Patent No. 6,159,443 to Hallahan, which is incorporated herein by reference in its entirety.

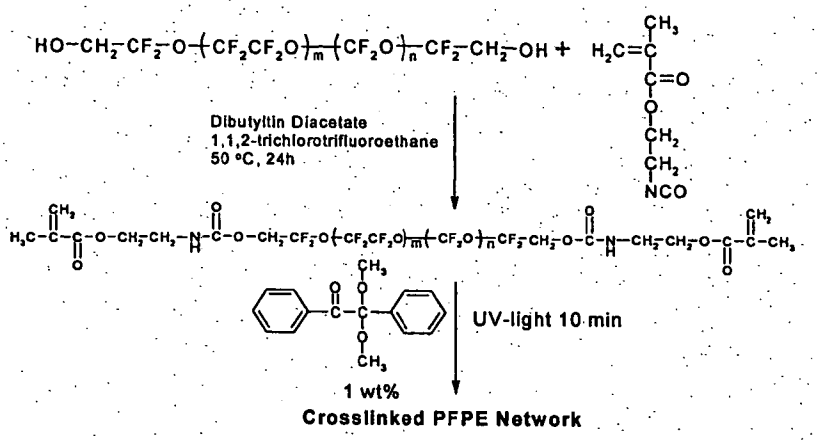
In sum, there exists a need in the art to identify new materials for use in imprint lithographic techniques. More particularly, there is a need in the art for methods for the fabrication of structures at the tens of micron level down to sub-100 nm feature sizes.

SUMMARY

The presently disclosed subject matter describes the use of fluorinated elastomer-based materials, in particular perfluoropolyether (PFPE)-based materials, in high-resolution soft or imprint lithographic applications, such as

micro- and nanoscale replica molding, and the first nano-contact molding of organic materials to generate high fidelity features using an elastomeric mold. Accordingly, the presently disclosed subject matter describes a method for producing free-standing, isolated nanostructures of any shape using soft or imprint lithography techniques.

A photocurable liquid PFPE exhibits ideal properties for soft lithography. A representative scheme for the synthesis and photocuring of functional perfluoropolyethers is provided in Scheme 1.



Scheme 1. Synthesis and Photocuring of Functional Perfluoropolyethers.

This PFPE material a low surface energy (about 12 mN/m), is non-toxic, UV transparent, highly gas permeable, and cures into a tough, durable, highly fluorinated elastomer with excellent release properties and resistance to swelling. The properties of these materials can be tuned over a wide range through the judicious choice of additives, fillers, reactive co-monomers, and functionalization agents. Such properties that are desirable to modify, include, but are not limited to, modulus, tear strength, surface energy, permeability, functionality, mode of cure, solubility and swelling characteristics, and the like. The non-swelling nature and easy release properties of the presently disclosed PFPE materials allows for nanostructures to be fabricated from any material. Further, the presently disclosed subject matter can be expanded to large scale rollers or conveyor

belt technology or rapid stamping that allow for the fabrication of nanostructures on an industrial scale.

The nanostructures described by the presently disclosed subject matter can be used in several applications, including, but not limited to, semiconductor manufacturing, especially used as molding etch barriers without scum layers for the fabrication of semiconductor devices; crystals; materials for displays; photovoltaics; optoelectronic devices; routers; gratings; radio frequency identification (RFID) devices; catalysts; fillers and additives; detoxifying agents; etch barriers; atomic force microscope (AFM) tips; parts for nano-machines; the delivery of a therapeutic agent, such as a drug or genetic material; cosmetics; chemical mechanical planarization (CMP) particles; and porous particles and shapes of any kind that will enable the nanotechnology industry.

Certain objects of the presently disclosed subject matter having been stated hereinabove, which are addressed in whole or in part by the presently disclosed subject matter, other aspects and objects will become evident as the description proceeds when taken in connection with the accompanying Examples as best described herein below.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic illustration of the presently disclosed method for forming freestanding nanostructures (jagged edges indicate a continuing structure).

Figure 2 is a schematic illustration of the presently disclosed method for fabricating isolated spherical nanoparticles.

Figure 3 is a schematic representation of the presently disclosed method for fabricating charged polymeric particles. (A) represents the electrostatic charging of the molded particle during polymerization or crystallization; (B) represents a charged nano-disc; (C) represents typical random juxtapositioning of uncharged nano-discs; and (D) represents the spontaneous aggregation of charged nano-discs into chain-like structures.

Figure 4 is a schematic illustration of multilayer particles that can be formed using the presently disclosed soft lithography method.

Figure 5 is a schematic representation of a (A) the presently disclosed method for making three dimensional nanostructures using a soft lithography technique; and (B) an exemplary structure made by the presently disclosed soft lithography method.

Figure 6 is a schematic representation of imprint lithography processes resulting in a "scum layer."

Figure 7 is a schematic representation of the presently disclosed imprint lithography method, which eliminates the "scum layer" by using a functionalized, non-wetting substrate and a non-wetting stamp.

DETAILED DESCRIPTION

The presently disclosed subject matter will now be described more fully hereinafter with reference to the accompanying Examples, in which representative embodiments are shown. The presently disclosed subject matter can, however, be embodied in different forms and should not be construed as limited to the embodiments set forth herein. Rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the embodiments to those skilled in the art.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this presently described subject matter belongs. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

Throughout the specification and claims, a given chemical formula or name shall encompass all optical and stereoisomers, as well as racemic mixtures where such isomers and mixtures exist.

1. Formation of Isolated Nanostructures

In some embodiments, the presently disclosed subject matter provides a method for making isolated nanostructures. Turning now to Figure 1, in some embodiments, the process comprises an etched substrate, such as a silicon wafer, which is etched in the desired pattern to form a patterned

master. See Figure 1A. A liquid fluoropolymer composition, such as a PFPE-based precursor, is then poured onto the etched substrate and cured, for example through exposure to UV light, thereby curing it in the desired pattern. See Figure 1B. The cured PFPE is then removed from the etched substrate to form a patterned stamp. See Figure 1C.

Separately, a substrate, such as a silicon wafer, is treated or is coated with a non-wetting material. See Figure 1D. In some embodiments, the non-wetting material comprises a PFPE elastomer that can be further exposed to UV light and cured to form a thin, non-wetting layer on the surface of the substrate. In some embodiments, the substrate is made non-wetting to the liquid by treating the substrate with a small molecule, such as an alkyl- or fluoroalkyl-silane. A droplet of curable resin, monomer, or solution in which the desired nanostructures will be formed is then placed on the coated wafer. See Figure 1D. The patterned stamp formed in Step 1C is then brought into contact with the droplet so that it takes the desired pattern. See Figure 1E. Without being bound to any particular theory, once pressure is applied, the affinity of the PFPE stamp for the non-wetting coating or surface treatment on the substrate in combination with the non-wetting behavior of the PFPE-based template material and surface treated or coated substrate causes the liquid to be excluded from all areas except for those inside the pattern. See Figure 1F.

Further, in embodiments essentially free of non-wetting or low wetting materials with which to sandwich the small drop of the curable resin, monomer, or solution, a "scum" layer that interconnects the objects being stamped forms. The resin/monomer can then be photocured through the stamp or thermally cured while under pressure.

In some embodiments, a material, including but not limited to a polymer, an organic compound, or an inorganic compound, can be dissolved in a solvent, patterned, and the solvent can be released. See Figure 1F.

Once the material is patterned, the entire stamp is removed from the coated substrate. See Figure 1G. The cured nanostructures are confined to the patterned areas of the stamp. See Figure 1G. The structures could be

retained on the substrate in defined regions once the stamp is removed. This embodiment would be desirable for use in the manufacture of semiconductor devices where essentially scum-layer free features could be used as etch barriers or as conductive, semiconductive, or dielectric layers directly, mitigating or reducing the need to use traditional and expensive photolithographic processes. In addition, the structures can be removed from the substrate or from the stamp by a variety of methods which include but are not limited to: (1) reapplying the stamp to a surface that has an affinity for the nanostructures; (2) deforming the stamps, or using other mechanical methods, including sonication, in such a manner that the structures are naturally released from the stamp; (3) swelling the stamp reversibly with supercritical carbon dioxide or another solvent that will extrude the structures; and (4) washing the stamp with a solvent that has an affinity for the nanostructures and will wash them out of the patterned stamp. See Figure 1H.

More particularly, in some embodiments, a method for preparing a patterned structure is disclosed, the method comprising:

(a) disposing a volume of liquid material between two surface elements, wherein at least one of the two surface elements comprises a patterned area;

(b) contacting the two surface elements by applying contact pressure to create a point of contact between the two surface elements and confine the liquid material to the patterned area of at least one surface element;

(c) forming a structure within the patterned area from the liquid material of at least one surface element; and

(d) removing at least one of the surface elements to provide a structure.

In some embodiments, the patterned area comprises a nanoscale patterned area. In some embodiments, the structure comprises a nanoscale structure.

In some embodiments, at least one of the surface elements comprises an elastomeric material. In some embodiments, at least one of the surface elements comprises a patterned polymeric stamp. In some embodiments, the patterned polymeric stamp is prepared by a method comprising:

(a) providing a patterned substrate;

(b) contacting the patterned substrate with a polymer precursor;

(c) curing the polymer precursor to form a patterned polymeric stamp comprising patterned areas; and

(d) removing the patterned polymeric stamp from the patterned substrate.

In some embodiments, the patterned substrate comprises an etched substrate. In some embodiments, the etched substrate comprises an etched silicon wafer. In some embodiments, the patterned substrate comprises a nanoscale patterned substrate.

In some embodiments, the polymer precursor comprises a fluoropolymer precursor. In some embodiments, the fluoropolymer precursor comprises a liquid perfluoropolyether precursor. In some embodiments, the curing of the polymer precursor is performed by exposing the polymer precursor to actinic radiation. In some embodiments, the actinic radiation is selected from one of thermal and electromagnetic radiation. In some embodiments, the actinic radiation comprises electromagnetic radiation. In some embodiments, the electromagnetic radiation comprises ultraviolet radiation.

In some embodiments, the patterned polymeric stamp comprises a gas-permeable or solvent permeable polymer. In some embodiments, the patterned polymeric stamp comprises a crosslinked, fluorinated polymer. In some embodiments, the crosslinked, fluorinated polymer comprises a crosslinked perfluoropolyether-derived polymer. In some embodiments, other reactive co-monomers or modifiers were used or added to modify the properties of the polymeric stamp. In some embodiments, the patterned polymeric stamp comprises an optically transparent polymer.

In some embodiments, the patterned area of the at least one of the surface elements comprises structural features. In some embodiments, the

structural features range from about 10 microns to about 1 nanometer in size. In some embodiments, the structural features range from about 10 microns to about 1 micron in size. In some embodiments, the structural features range from about 1 micron to about 100 nm in size. In some embodiments, the structural features range from about 100 nm to about 1 nm in size. In some embodiments, the structural features comprise a channel.

In some embodiments, the liquid material is selected from one of a curable material and a solution. In some embodiments, the curable material is selected from one of a curable resin and a curable monomer. In some embodiments, the method further comprises curing the curable material while the material is confined to the patterned area (e.g., a nanoscale patterned area) of at least one of the surface elements.

In some embodiments, the solution comprises a solvent and a soluble material selected from the group consisting of a polymer, an organic compound, and an inorganic compound. In some embodiments, the solvent is removed while the soluble material is confined within the patterned area (e.g., a nanoscale patterned area) of at least one of the surface elements.

In some embodiments, at least one of the surface elements comprises a microelectronic device. In some embodiments, the microelectronic device comprises a silicon wafer.

In some embodiments, at least one of the surface elements is coated or treated with a non-wetting or low-wetting material. In some embodiments, the non-wetting or low-wetting material comprises a perfluoropolyether precursor. In some embodiments, the perfluoropolyether precursor is cured to form a thin layer of perfluoropolyether elastomer on at least one of the surface elements. In some embodiments, the curing of the perfluoropolyether precursor is performed by one of exposing the perfluoropolyether precursor to ultraviolet radiation and thermally curing the perfluoropolyether precursor. In some embodiments, the non-wetting or low-wetting treatment comprises a silane.

In some embodiments, the structure comprises a nanostructure. In some embodiments, the nanostructure comprises a free standing nanostructure.

2. Formation of Spherical Particles Through "Liquid Reduction"

In some embodiments, the presently disclosed subject matter provides a "liquid reduction" process for forming spherical particles. Without being bound to any particular theory, the non-wetting nature of the presently disclosed PFPE materials allows for the generation of spherical particles. This process is illustrated in Figure 2. The patterned stamp is placed on top of a drop of curable liquid, which is in contact with a substrate. The liquid then enters the channels of the patterned stamp, but a "scum layer" remains between the stamp and substrate. A pressue is applied to the stamp, thereby forming a contact point between the stamp and the substrate and eliminating the scum layer. A higher pressure is then applied to the stamp, thereby forming smaller liquid particles inside the channels and forcing more liquid out of the channels. The original contact pressure is then applied. The gas permeable nature of the stamp alllows for part of the void to be filled with nitrogen or another gas, thereby leaving a spherical droplet. Once this liquid reduction is achieved, the particles are cured in their spherical shape and removed from the stamp.

More particularly, in some embodiments, the method comprises:

(a) reducing the volume of the liquid material confined in the stamp by one of:

(i) applying additional contact pressure to at least one of the surface elements to reduce the volume of the liquid material formed within the patterned area of at least one of the surface elements and extrude additional liquid material from the patterned area; and

(ii) allowing some of the liquid or solvent of a multicomponent system to evaporate or permeate through the stamp;

(b) reducing the additional contact pressure from the surface element;

(c) introducing gas within the patterned area of at least one of the surface elements;

(d) forming at least one or more structures within the patterned area of at least one of the surface elements; and

(e) releasing the structure or structures from the patterned area of at least one of the surface elements.

In some embodiments, the structures are particles, for example spherical particles. In some embodiments, the particles comprise monodisperse particles. In some embodiments, the particles are nanoscale particles, and the nanoscale particles can comprise a spherical shape. In some embodiments, the nanoscale particles can comprise monodisperse nanoscale particles. In some embodiments, the method further comprises applying an electric field during the forming of the particles to provide a charged polymeric particle.

In some embodiments, the presently disclosed subject matter describes a method for stamping out polymeric nano- to micro-electrets by applying an electric field during the polymerization and/or crystallization step during molding (**A** in Figure 3) to yield a charged polymeric particle (**B** in Figure 3). In some embodiments, the charged polymeric particles spontaneously aggregate into chain-like structures (**D** in Figure 3) instead of the random configurations shown in **C** of Figure 3.

In some embodiments, the charged polymeric particle comprises a polymeric electret. In some embodiments, the polymeric electret comprises a polymeric nano-electret. In some embodiments, the charged polymeric particles aggregate into chain-like structures. In some embodiments, the charged polymeric particles comprise an additive for an electro-rheological device. In some embodiments, the electro-rheological device is selected from the group consisting of clutches and active dampening devices. In some embodiments, the charged polymeric particles comprise nano-piezoelectric devices. In some embodiments, the nano-piezoelectric devices are selected from the group consisting of actuators, switches, and mechanical sensors.

3. Formation of Multilayer Structures

In some embodiments, the presently disclosed subject matter provides a process for forming multilayer structures, including multilayer particles. In some embodiments, the multilayer structures, including multilayer particles, comprise nanoscale multilayer structures. In some embodiments, multilayer structures are formed by depositing multiple thin layers of immisible liquids and/or solutions onto a substrate and forming particles as described by any of the methods hereinabove. The immiscibility of the liquid can be based on any physical characteristic, including but not limited to density, polarity, and volatility. Examples of possible morphologies of the presently disclosed subject matter are illustrated in Figure 4 and include, but are not limited to, multi-phase sandwich stuctures, core-shell particles, and internal emulsions, microemulsions and/or nano-sized emulsions.

More particularly, in some embodiments, the method comprises disposing a plurality of immiscible liquids between the two surface elements to form a multilayer structure, e.g., a multilayer nanostructure. In some embodiments, the multilayer structure comprises a multilayer particle. In some embodiments, the multilayer structure comprises a structure selected from the group consisting of multi-phase sandwich structures, core-shell particles, internal emulsions, microemulsions, and nanosized emulsions.

4. Fabrication of Complex Multidimensional Structures

In some embodiments, the currently disclosed subject matter provides a process for fabricating complex, multidimensional structures. In some embodiments, complex multidimensional structures can be formed by performing the process illustrated in Figure 1. In some embodiments, the process comprises imprinting onto a patterned substrate that is aligned with the patterned stamp (instead of imprinting onto a smooth substrate) to generate isolated multidimensional structures that are cured and released as described hereinabove. An illustration of the process for forming complex multidimensional structures and examples of such structures is provided in Figure 5.

More particularly, in some embodiments, both surface elements comprise a patterned area. In some embodiments, the method further comprises aligning the patterned area of the surface elements before applying contact pressure to at least one of the surface elements to form a patterned structure. In some embodiments, the patterned structure comprises a nanoscale patterned structure. In some embodiments, the patterned structure comprises a multidimensional structure. In some embodiments, the multidimensional structure comprises a nanoscale multidimensional structure. In some embodiments, the multidimensional structure comprises a plurality of structural features. In some embodiments, the structural features comprise a plurality of heights. In some embodiments, a microelectronic device produced by the method is described herein. With this technique any structures imaginable, including "dual damscene" structures for microelectronics can be generated. In some embodiments, the microelectronic device is selected from the group consisting of integrated circuits, semiconductor particles, quantum dots, and dual damascene structures. In some embodiments, the microelectronic device exhibits certain physical properties selected from the group consisting of etch resistance, low dielectric constant, high dielectric constant, conducting, semiconducting, insulating, porosity, and non-porosity.

5. Imprint Lithography Free of a Residual "Scum Layer"

A characteristic of imprint lithography that has restrained its full potential is the formation of a "scum layer" once the resin is patterned. The "scum layer" comprises residual resin that remains between the stamp and the substrate. See Figure 6. In some embodiments, the presently disclosed subject matter provides a process for using imprint lithography to generate patterns essentially free of a scum layer. See Figure 7. The substrate is functionalized with a non-wetting material. The non-wetting material further comprises functional groups that bind to the curable resin. The non-wetting material includes, but is not limited to, a mixture of trichloro or trialkoxy silanes, or a single trichloro or trialkoxy silane comprising non-wetting and

reactive functional groups. Without being bound to any particular theory, the non-wetting nature of both the stamp and the substrate will drive the resin out except in patterned areas. Upon curing, the functional groups of the substrate react with the resin and bind it to the substrate. The substrate can be etched with traditional lithographic techniques.

More particularly, in some embodiments, at least one of the surface elements comprises a functionalized surface element. In some embodiments, the functionalized surface element is functionalized with a non-wetting material. In some embodiments, the non-wetting material comprises functional groups that bind to the liquid material. In some embodiments, the non-wetting material is selected from the group consisting of a trichloro silane, a trialkoxy silane, a trichloro silane comprising non-wetting and reactive functional groups, a trialkoxy silane comprising non-wetting and reactive functional groups, and mixtures thereof.

In some embodiments, the point of contact between the two surface elements is free of liquid material. In some embodiments, the point of contact between the two surface elements comprises residual liquid material. In some embodiments, the height of the residual liquid material is less than 30% of the height of the structure. In some embodiments, the height of the residual liquid material is less than 20% of the height of the structure. In some embodiments, the height of the residual liquid material is less than 10% of the height of the structure. In some embodiments, the height of the residual liquid material is less than 5% of the height of the structure. In some embodiments, the volume of liquid material is less than the volume of a patterned area. In some embodiments, substantially all of the volume of liquid material is confined to a patterned area of at least one of the surface elements. In some embodiments, having the point of contact between the two surface elements free of liquid material retards slippage between the two surface elements.

6. Removing the Patterned Structure from the Surface Element

In some embodiments, the removing of the patterned structure (e.g., a patterned nanostructure) from at least one of the surface elements is performed by one of:

(a) reapplying the surface element containing the patterned structure to a surface that has an affinity for the patterned structure;

(b) deforming the surface element containing the patterned structure such that the patterned structure is released from the surface element;

(c) swelling the surface element containing the patterned structure with a first solvent to extrude the patterned structure; and

(d) washing the surface element containing the patterned structure with a second solvent that has an affinity for the patterned structure.

In some embodiments, the first solvent comprises supercritical fluid carbon dioxide. In some embodiments, the first solvent comprises water. In some embodiments, the first solvent comprises an aqueous solution comprising water and a detergent. In embodiments, the deforming the surface element is performed by applying a mechanical force to the surface element. In some embodiments, the method of removing the patterned structure further comprises a sonication method.

7. Methods of Forming a Pattern on a Substrate or a Surface

In some embodiments, the method of forming a pattern on a substrate comprises:

(a) providing a template, wherein the template comprises a first surface and a plurality of recesses extending from the first surface toward an opposing second surface and a plurality of non-recessed areas, wherein the plurality of recesses and non-recessed areas defines a plurality of structural features in the first surface of the template;

(b) positioning the template and the substrate in a spaced relationship to each other so that a gap is created between the template and the substrate;

(c) disposing a volume of curable liquid in the gap between the template and the substrate;

(d) contacting the template with the volume of curable liquid;

(e) curing the curable liquid; and

(f) removing the template from the cured curable liquid to provide a pattern on a substrate.

In some embodiments, the template comprises an elastomeric material. In some embodiments, the template consists of an elastomeric material. In some embodiments, the template is an elastomeric material. In some embodiments, the elastomeric material comprises a crosslinked, fluorinated polymer. In some embodiments, the crosslinked, fluorinated polymer comprises a crosslinked, perfluoropolyether derivative. In some embodiments, the template comprises a template prepared by a soft lithography or by an imprint lithography technique.

In some embodiments, the volume of curable liquid fills the gap between the template and the substrate. In some embodiments, the volume of liquid fills the plurality of recesses of the template. In some embodiments, the template and the substrate comprise non-wetting materials with respect to the curable liquid material. In some embodiments, the contacting the template with the volume of curable liquid further comprises contacting the non-recessed areas of the template with the substrate, thereby eliminating all of the volume of curable liquid between the non-recessed areas of the template and the substrate. In some embodiments, essentially no scum layer is formed.

In some embodiments, a method of making a microelectronic device by employing the patterned substrate is disclosed. In some embodiments, the microelectronic device comprises a semiconductor.

In some embodiments, a method of making a particle by employing the patterned substrate is disclosed. In some embodiments, the particle further comprises a therapeutic agent. In some embodiments, the therapeutic agent

comprises a drug. In some embodiments, the therapeutic agent comprises genetic material. In some embodiments, the genetic material is selected from the group consisting of a non-viral gene vector, DNA, RNA, RNAi, and a viral particle.

In some embodiments, a method of making a display device by employing the patterned substrate is disclosed. In some embodiments, the display device comprises an Organic Light Emitting Diode. In some embodiments, the Organic Light Emitting Diode comprises a patterning Organic Light Emitting Diode.

In some embodiments, a method of forming a pattern on a surface is disclosed, the method comprising selectively exposing the surface of an article to an agent by:

(a) shielding a first portion of the surface of the article with a masking system, wherein the masking system comprises a elastomeric mask in conformal contact with the surface of the article; and

(b) applying an agent to be patterned within the masking system to a second portion of the surface of the article, while preventing application of the agent to the first portion shielded by the masking system.

In some embodiments, the elastomeric mask comprises a plurality of channels. In some embodiments, each of the channels has a cross-sectional dimension of less than about 1 millimeter. In some embodiments, each of the channels has a cross-sectional dimension of less than about 1 micron. In some embodiments, each of the channels has a cross-sectional dimension of less than about 100 nm. In some embodiments, each of the channels has a cross-sectional dimension of about 1 nm. In some embodiments, the agent swells the elastomeric mask less than 25%.

In some embodiments, the agent comprises an organic electroluminescent material or a precursor thereof. In some embodiments, the method further comprising allowing the organic electroluminescent material to form from the agent at the second portion of the surface, and

establishing electrical communication between the organic electroluminescent material and an electrical circuit.

In some embodiments, the agent comprises a liquid or is carried in a liquid. In some embodiments, the agent comprises the product of chemical vapor deposition. In some embodiments, the agent comprises a product of deposition from a gas phase. In some embodiments, the agent comprises a product of e-beam deposition, evaporation, or sputtering. In some embodiments, the agent comprises a product of electrochemical deposition. In some embodiments, the agent comprises a product of electroless deposition. In some embodiments, the agent is applied from a fluid precursor. In some embodiments, comprises a solution or suspension of an inorganic compound. In some embodiments, the inorganic compound hardens on the second portion of the article surface.

In some embodiments, the fluid precursor comprises a suspension of particles in a fluid carrier. In some embodiments, the method further comprises allowing the fluid carrier to dissipate thereby depositing the particles at the first region of the article surface. In some embodiments, the fluid precursor comprises a chemically active agent in a fluid carrier. In some embodiments, the method further comprises allowing the fluid carrier to dissipate thereby depositing the chemically active agent at the first region of the article surface.

In some embodiments, the chemically active agent comprises a polymer precursor. In some embodiments, the method further comprises forming a polymeric article from the polymer precursor. In some embodiments, the chemically active agent comprises an agent capable of promoting deposition of a material. In some embodiments, the chemically active agent comprises an etchant. In some embodiments, the method further comprises allowing the second portion of the surface of the article to be etched. In some embodiments, the method further comprises removing the elastomeric mask of the masking system from the first portion of the article surface while leaving the agent adhered to the second portion of the article surface.

8. Method of Delivering a Therapeutic Agent

In some embodiments, a method of delivering a therapeutic agent to a target is disclosed, the method comprising:

(a) providing a particle produced by a method comprising:

(i) disposing a volume of liquid material between two surface elements, wherein at least one of the two surface elements comprises a patterned area;

(ii) contacting the two surface elements by applying contact pressure to create a point of contact between the two surface elements and confine the liquid material to the patterned area of at least one surface element;

(iii) forming at least one or more particles within the patterned area of at least one of the surface elements; and

(iv) releasing the particles from the patterned area of at least one of the surface elements.

(b) admixing the therapeutic agent with the particle; and

(c) delivering the particle comprising the therapeutic agent to the target.

In some embodiments, the particle has a diameter of less than 100 microns. In some embodiments, the particle has a diameter of less than 10 microns. In some embodiments, the particle has a diameter of less than 1 micron. In some embodiments, the particle has a diameter of less than 100 nm. In some embodiments, the particle has a diameter of less than 10 nm. In some embodiments, the therapeutic agent is selected from one of a drug and genetic material, such as a non-viral gene vector, DNA, RNA, RNAi, and a viral particle. In some embodiments, the particle comprises a biodegradable polymer. In some embodiments, the biodegradable polymer is selected from the group consisting of a polyester, a polyanhydride, a polyamide, a phosphorous-based polymer, a poly(cyanoacrylate), a polyurethane, a polyorthoester, a polydihydropyran, and a polyacetal. In some embodiments, the polyester is selected from the group consisting of polylactic acid, polyglycolic acid, poly(hydroxybutyrate), poly(ε-caprolactone), poly(β-malic acid), and poly(dioxanones). In some embodiments, the

polyanhydride is selected from the group consisting of poly(sebacic acid), poly(adipic acid), and poly(terpthalic acid). In some embodiments, the polyamide is selected from the group consisting of poly(imino carbonates) and polyaminoacids. In some embodiments, the phosphorous-based polymer is selected from the group consisting of polyphosphates, polyphosphonates, and polyphosphazenes. In some embodiments, the polymer is responsive to stimuli, such as pH, light or temperature. Responses to such stimuli can include swelling, which can facilitate release of its cargo, or degradation.

Examples

The following Examples have been included to provide guidance to one of ordinary skill in the art for practicing representative embodiments of the presently disclosed subject matter. In light of the present disclosure and the general level of skill in the art, those of skill can appreciate that the following Examples are intended to be exemplary only and that numerous changes, modifications, and alterations can be employed without departing from the scope of the presently disclosed subject matter.

1. Representative Procedure for Synthesis and Curing Photocurable Perfluoropolyethers

In some embodiments, the synthesis and curing of PFPE materials of the presently disclosed subject matter is performed by using the method described by Rolland, J. P., et al., *J. Am. Chem. Soc.*, **2004**, *126*, 2322-2323. Briefly, this method involves the methacrylate-functionalization of a commercially available PFPE diol (M_n = 3800 g/mol) with isocyanatoethyl methacrylate. Subsequent photocuring of the material is accomplished through blending with 1 wt% of 2,2-dimethoxy-2-phenylacetophenone and exposure to UV radiation (λ = 365 nm).

More particularly, in a typical preparation of perfluoropolyether dimethacrylate (PFPE DMA), poly(tetrafluoroethylene oxide-*co*-difluoromethylene oxide)α,ω diol (ZDOL, average M_n *ca.* 3,800 g/mol, 95%, Aldrich Chemical Company, Milwaukee, Wisconsin, United States of America) (5.7227g, 1.5 mmol) was added to a dry 50 mL round bottom flask and

purged with argon for 15 minutes. 2-isocyanatoethyl methacrylate (EIM, 99%, Aldrich) (0.43 mL, 3.0 mmol) was then added via syringe along with 1,1,2-trichlorotrifluoroethane (Freon 113 99%, Aldrich) (2 mL), and dibutyltin diacetate (DBTDA, 99%, Aldrich) (50 µL). The solution was immersed in an oil bath and allowed to stir at 50 °C for 24 h. The solution was then passed through a chromatographic column (alumina, Freon 113, 2 x 5 cm). Evaporation of the solvent yielded a clear, colorless, viscous oil, which was further purified by passage through a 0.22-µm polyethersulfone filter.

In a representative curing procedure for PFPE DMA, 1 wt% of 2,2-dimethoxy-2-phenyl acetophenone (DMPA, 99% Aldrich), (0.05g, 2.0 mmol) was added to PFPE DMA (5g, 1.2 mmol) along with 2 mL Freon 113 until a clear solution was formed. After removal of the solvent, the cloudy viscous oil was passed through a 0.22-µm polyethersulfone filter to remove any DMPA that did not disperse into the PFPE DMA. The filtered PFPE DMA was then irradiated with a UV source (Electro-Lite Corporation, Danbury, Connecticut, United States of America, UV curing chamber model no. 81432-ELC-500, λ = 365 nm) while under a nitrogen purge for 10 min. This resulted in a clear, slightly yellow, rubbery material.

2. Representative Fabrication of a PFPE DMA Device

In some embodiments, a PFPE DMA device, such as a stamp, was fabricated according to the method described by Rolland, J. P., et al., *J. Am. Chem. Soc.*, **2004**, *126*, 2322-2323. Briefly, the PFPE DMA containing a photoinitiator, such as DMPA, was spin coated (800 rpm) to a thickness of 20 µm onto a Si wafer containing the desired photoresist pattern. This coated wafer was then placed into the UV curing chamber and irradiated for 6 seconds. Separately, a thick layer (about 5 mm) of the material was produced by pouring the PFPE DMA containing photoinitiator into a mold surrounding the Si wafer containing the desired photoresist pattern. This wafer was irradiated with UV light for one minute. Following this, the thick layer was removed. The thick layer was then placed on top of the thin layer such that the patterns in the two layers were precisely aligned, and then the

entire device was irradiated for 10 minutes. Once complete, the entire device was peeled from the Si wafer with both layers adhered together.

It will be understood that various details of the presently disclosed subject matter can be changed without departing from the scope of the presently disclosed subject matter. Furthermore, the foregoing description is for the purpose of illustration only, and not for the purpose of limitation.

CLAIMS

What is claimed is:

1. A method for preparing a patterned structure, the method comprising:
   (a) disposing a volume of liquid material between two surface elements, wherein at least one of the two surface elements comprises a patterned area;
   (b) contacting the two surface elements by applying contact pressure to create a point of contact between the two surface elements and confine the liquid material to the patterned area of at least one surface element;
   (c) forming a structure within the patterned area from the liquid material of at least one surface element; and
   (d) removing at least one of the surface elements to provide a structure.
2. The method of Claim 1, wherein at least one of the surface elements comprises an elastomeric material.
3. The method of Claim 1, wherein the patterned area comprises a nanoscale patterned area.
4. The method of Claim 1, wherein at least one of the surface elements comprises a patterned polymeric stamp.
5. The method of Claim 4, wherein the patterned polymeric stamp is prepared by the method comprising:
   (a) providing a patterned substrate;
   (b) contacting the patterned substrate with a polymer precursor;
   (c) curing the polymer precursor to form a patterned polymeric stamp comprising patterned areas; and
   (d) removing the patterned polymeric stamp from the patterned substrate.
6. The method of Claim 5, wherein the patterned substrate comprises a nanoscale patterned substrate.
7. The method of Claim 5, wherein the patterned substrate comprises an etched substrate.

8. The method of Claim 7, wherein the etched substrate comprises an etched silicon wafer.

9. The method of Claim 5, wherein the polymer precursor comprises a fluoropolymer precursor.

10. The method of Claim 9, wherein the fluoropolymer precursor comprises a liquid perfluoropolyether precursor.

11. The method of Claim 5, wherein the curing of the polymer precursor is performed by exposing the polymer precursor to actinic radiation.

12. The method of Claim 11, wherein the actinic radiation is selected from one of thermal and electromagnetic radiation.

13. The method of Claim 12, wherein the actinic radiation comprises electromagnetic radiation.

14. The method of Claim 13, wherein the electromagnetic radiation comprises ultraviolet radiation.

15. The method of Claim 5, wherein the patterned polymeric stamp comprises a gas-permeable polymer.

16. The method of Claim 5, wherein the patterned polymeric stamp comprises a solvent-permeable polymer.

17. The method of Claim 5, wherein the patterned polymeric stamp comprises a crosslinked, fluorinated polymer.

18. The method of Claim 17, wherein the crosslinked, fluorinated polymer comprises a crosslinked perfluoropolyether polymer.

19. The method of Claim 17, wherein the crosslinked, fluorinated polymer comprises a modified crosslinked, fluorinated polymer.

20. The method of Claim 19, wherein a co-monomer or modifier is employed to modify the crosslinked, fluorinated polymer.

21. The method of Claim 5, wherein the patterned polymeric stamp comprises an optically transparent polymer.

22. The method of Claim 1, wherein the patterned area of the at least one of the surface elements comprise structural features.

23. The method of Claim 22, wherein the structural features range from about 10 microns to about 1 nanometer in size.

24. The method of Claim 23, wherein the structural features range from about 10 microns to about 1 micron in size.

25. The method of Claim 23, wherein the structural features range from about 1 micron to about 100 nm in size.

26. The method of Claim 23, wherein the structural features range from about 100 nm to about 1 nm in size.

27. The method of Claim 22, wherein the structural features comprise a channel.

28. The method of Claim 1, wherein the liquid material is selected from one of a curable material and a solution.

29. The method of Claim 28, wherein the curable material is selected from one of a curable resin and a curable monomer.

30. The method of Claim 29, further comprising curing the curable material while the material is confined to the patterned area of at least one of the surface elements.

31. The method of Claim 28, wherein the solution comprises a solvent and a soluble material, wherein the soluble material is selected from the group consisting of a polymer, an organic compound, and an inorganic compound.

32. The method of Claim 31, wherein the solvent is removed while the soluble material is confined within the patterned area of at least one of the surface elements.

33. The method of Claim 1, wherein at least one of the surface elements comprises a microelectronic device.

34. The method of Claim 33, wherein the microelectronic device comprises a silicon wafer.

35. The method of Claim 1, wherein at least one of the surface elements is treated or coated with a non-wetting or low-wetting material.

36. The method of Claim 35, wherein the non-wetting or low-wetting material comprises a perfluoropolyether precursor.

37. The method of Claim 36, wherein the perfluoropolyether precursor is cured to form a thin layer of perfluoropolyether elastomer on at least one of the surface elements.

38. The method of Claim 37, wherein the curing of the perfluoropolyether precursor is performed by one of exposing the perfluoropolyether precursor to ultraviolet radiation and thermally curing the perfluoropolyether precursor.

39. The method of Claim 35, wherein the non-wetting or low-wetting material is selected from one of an alkylsilane and a fluoroalkylsilane.

40. A structure produced by the method of Claim 1.

41. The method of Claim 1, wherein the structure comprises a nanostructure.

42. A nanostructure produced by the method of Claim 41.

43. The method of Claim 1, wherein the structure comprises a free standing structure.

44. A freestanding structure produced by the method of Claim 43.

45. The method of Claim 43, wherein the free standing structure comprises a nanostructure.

46. The method of Claim 1, further comprising disposing a plurality of immiscible liquids between the two surface elements.

47. The method of Claim 1, wherein the structure further comprises a multilayer structure.

48. The method of Claim 47, wherein the multilayer structure comprises a nanoscale multilayer structure.

49. The method of Claim 47, wherein the multilayer structure comprises a multilayer particle.

50. The method of Claim 49, wherein the multilayer particle comprises a nanoscale multilayer particle.

51. The method of Claim 47, wherein the multilayer structure comprises a structure selected from the group consisting of multi-phase sandwich structures, core-shell particles, internal emulsions, microemulsions, and nanosized emulsions.

52. A multilayer structure produced by the method of Claim 47.

53. A multilayer particle produced by the method of Claim 49.

54. The method of Claim 1, further comprising:

(a) reducing the volume of the liquid material confined to at least one of the surface elements by one of:

(i) applying additional contact pressure to at least one of the surface elements to reduce the volume of the liquid material formed within the patterned area of at least one of the surface elements and extrude additional liquid material from the patterned area; and

(ii) allowing some of the liquid to evaporate or permeate through the surface element;

(b) reducing the additional contact pressure from the surface element;

(c) introducing gas within the patterned area of at least one of the surface elements;

(d) forming at least one or more structures within the patterned area of at least one of the surface elements; and

(e) releasing the structure or structures from the patterned area of at least one of the surface elements.

55. The method of Claim 54, wherein the structures comprise particles.

56. The method of Claim 55, wherein the particles comprise a spherical shape.

57. The method of Claim 55, wherein the particles comprise monodisperse particles.

58. The method of Claim 55, wherein the particles comprise nanoscale particles.

59. The method of Claim 54, further comprising applying an electric field during the forming of the structure or structures within the patterned area of at least one of the surface elements to form charged polymeric particles.

60. The method of Claim 59, wherein the charged polymeric particles comprise polymeric electrets.

61. The method of Claim 60, wherein the polymeric electrets comprise polymeric nano-electrets.

62. The method of Claim 59, wherein the charged polymeric particles aggregate into chain-like structures.

63. The method of Claim 59, wherein the charged polymeric particles comprise an additive for an electro-rheological device.

64. The method of Claim 63, wherein the electro-rheological device is selected from the group consisting of clutches and active dampening devices.

65. The method of Claim 59, wherein the charged polymeric particles comprise nano-piezoelectric devices.

66. The method of Claim 65, wherein the nano-piezoelectric devices are selected from the group consisting of actuators, switches, and mechanical sensors.

67. A particle produced by the method of Claim 54.

68. Monodisperse particles produced by the method of Claim 54.

69. A charged polymeric particle produced by the method of Claim 59.

70. A polymeric electret produced by the method of Claim 60.

71. A polymeric nano-electret produced by the method of Claim 61.

72. A nano-piezoelectric device produced by the method of Claim 65.

73. The method of Claim 1, wherein both surface elements comprise a patterned area.

74. The method of Claim 73, further comprising aligning the patterned areas of the surface elements before applying contact pressure to at least one of the surface elements to form a patterned structure.

75. The method of Claim 74, wherein the patterned structure comprises a multidimensional structure.

76. The method of Claim 75, wherein the multidimensional structure comprises a plurality of structural features.

77. The method of Claim 76, wherein the structural features comprise a plurality of heights.

78. The method of Claim 73, wherein the patterned area comprises a patterned nanoscale area.

79. The method of Claim 74, wherein the patterned structure comprises a patterned nanoscale structure.

80. The method of Claim 75, wherein the multidimensional structure comprises a multidimensional nanoscale structure.

81. A microelectronic device produced by the method of Claim 74.

82. The method of Claim 81, wherein the microelectronic device is selected from the group consisting of integrated circuits, semiconductor particles, quantum dots, and dual damascene structures.

83. The method of Claim 81, wherein the microelectronic device exhibits a physical property selected from the group consisting of etch resistance, low dielectric constant, high dielectric constant, conducting, semiconducting, insulating, porosity, non-porosity, and combinations thereof.

84. The method of Claim 1, wherein at least one of the surface elements comprises a functionalized surface element.

85. The method of Claim 84, wherein the functionalized surface element is functionalized with a non-wetting material.

86. The method of Claim 85, wherein the non-wetting material comprises functional groups that bind to the liquid material.

87. The method of Claim 86, wherein the non-wetting material is selected from the group consisting of a trichloro silane, a trialkoxy silane, a trichloro silane comprising non-wetting and reactive functional groups, a trialkoxy silane comprising non-wetting and reactive functional groups, and mixtures thereof.

88. The method of Claim 1, wherein the point of contact between the two surface elements is free of liquid material.

89. The method of Claim 1, wherein the point of contact between the two surface elements comprises residual liquid material.

90. The method of Claim 89, wherein the residual liquid material has a height less than 30% of the height of the structure.

91. The method of Claim 89, wherein the residual liquid material has a height less than 20% of the height of the structure.

92. The method of Claim 89, wherein the residual liquid material has a height less than 10% of the height of the structure.

93. The method of Claim 89, wherein the residual liquid material has a height less than 5% of the height of the structure.

94. The method of Claim 1, wherein the volume of liquid material is less than the volume of the patterned area.

95. The method of Claim 94, wherein substantially all of the volume of liquid material is confined to the patterned area of at least one of the surface elements.

96. The method of Claim 95, wherein having the point of contact between the two surface elements free of liquid material retards slippage between the two surface elements.

97. The method of Claim 1, wherein the removing of the patterned structure from at least one of the surface elements is performed by one of:

(a) reapplying the surface element containing the structure to a second surface element that has an affinity for the patterned structure;

(b) deforming the surface element containing the structure such that the structure is released from the surface element;

(c) swelling the surface element containing the structure with a first solvent to extrude the structure; and

(d) washing the surface element containing the structure with a second solvent that has an affinity for the structure.

98. The method of Claim 97, wherein the first solvent is selected from the group consisting of supercritical fluid carbon dioxide, water, and an aqueous solution comprising water and a detergent.

99. The method of Claim 97, wherein the deforming the surface element is performed by applying a mechanical force to the surface element.

100. The method of Claim 97, further comprising a sonication method.

101. A method of forming a pattern on a substrate, the method comprising:

(a) providing a template, wherein the template comprises a first surface and a plurality of recesses extending from the first surface toward an opposing second surface and a plurality of non-recessed areas, wherein the plurality of recesses and non-

recessed areas defines a plurality of structural features in the first surface of the template;

(b) positioning the template and the substrate in a spaced relationship to each other so that a gap is created between the template and the substrate;

(c) disposing a volume of curable liquid in the gap between the template and the substrate;

(d) contacting the template with the volume of curable liquid;

(e) curing the curable liquid; and

(f) removing the template from the cured curable liquid to provide a pattern on a substrate.

102. The method of Claim 101, wherein the template is an elastomeric material.

103. The method of Claim 101, wherein the template comprises an elastomeric material.

104. The method of Claim 102 or 103, wherein the elastomeric material comprises a crosslinked, fluorinated polymer.

105. The method of Claim 104, wherein the crosslinked, fluorinated polymer comprises a crosslinked, perfluoropolyether derivative.

106. The method of Claim 101, wherein the template comprises a template prepared by one of a soft lithography technique and an imprint lithography technique.

107. The method of Claim 101, wherein the volume of curable liquid fills the gap between the template and the substrate.

108. The method of Claim 101, wherein the volume of liquid fills the plurality of recesses of the template.

109. The method of Claim 101, wherein the template and the substrate comprise non-wetting materials with respect to the curable liquid material.

110. The method of Claim 101, wherein the contacting the template with the volume of curable liquid further comprises contacting the non-recessed areas of the template with the substrate, thereby eliminating all of the

volume of curable liquid between the non-recessed areas of the template and the substrate.

111. The method of Claim 110, wherein essentially no scum layer is formed.

112. A patterned substrate produced by the method of Claim 101.

113. A method of making a microelectronic device by employing the patterned substrate of Claim 101.

114. The method of Claim 113, wherein the microelectronic device comprises a semiconductor.

115. A method of making a particle by employing the patterned substrate of Claim 112.

116. The method of Claim 115, wherein the particle further comprises a therapeutic agent.

117. The method of Claim 116, wherein the therapeutic agent is selected from one of a drug and genetic material.

118. The method of Claim 117, wherein the therapeutic agent comprises a drug.

119. The method of Claim 117, wherein the genetic material is selected from the group consisting of a non-viral gene vector, DNA, RNA, RNAi, and a viral particle.

120. The method of Claim 119, wherein the therapeutic agent comprises a non-viral gene vector.

121. The method of Claim 119, wherein the therapeutic agent comprises a viral particle.

122. A method of making a display device by employing the patterned substrate of Claim 112.

123. The method of Claim 122, wherein the display device comprises an Organic Light Emitting Diode.

124. The method of Claim 123, wherein the Organic Light Emitting Diode comprises a patterning Organic Light Emitting Diode.

125. A method of forming a pattern on a surface, the method comprising selectively exposing the surface of an article to an agent by:

(a) shielding a first portion of the surface of the article with a masking system, wherein the masking system comprises a

elastomeric mask in conformal contact with the surface of the article; and

(b) applying an agent to be patterned within the masking system to a second portion of the surface of the article, while preventing application of the agent to the first portion shielded by the masking system.

126. The method of Claim 125, wherein the elastomeric mask comprises a plurality of channels.

127. The method of Claim 126, wherein each of the channels has a cross-sectional dimension of less than about 1 millimeter.

128. The method of Claim 126, wherein each of the channels has a cross-sectional dimension of less than about 1 micron.

129. The method of Claim 126, wherein each of the channels has a cross-sectional dimension of less than about 100 nm.

130. The method of Claim 126, wherein each of the channels has a cross-sectional dimension of about 1 nm.

131. The method of Claim 125, wherein the agent swells the elastomeric mask less than 25%.

132. The method of Claim 125, wherein the agent comprises an organic electroluminescent material or a precursor thereof.

133. The method of Claim 132, further comprising:

(a) allowing the organic electroluminescent material to form from the agent at the second portion of the surface, and

(b) establishing electrical communication between the organic electroluminescent material and an electrical circuit.

134. The method of Claim 125, wherein the agent comprises a liquid or is carried in a liquid.

135. The method of Claim 125, wherein the agent comprises the product of chemical vapor deposition.

136. The method of Claim 125, wherein the agent comprises a product of deposition from a gas phase.

137. The method of Claim 125, wherein the agent comprises a product of e-beam deposition, evaporation, or sputtering.

138. The method of Claim 125, wherein the agent comprises a product of electrochemical deposition.

139. The method of Claim 125, wherein the agent comprises a product of electroless deposition.

140. The method of Claim 125, wherein the agent is applied from a fluid precursor.

141. The method of Claim 140, wherein the fluid precursor comprises a solution or suspension of an inorganic compound.

142. The method of Claim 141, wherein the inorganic compound hardens on the second portion of the article surface.

143. The method of Claim 140, wherein the fluid precursor comprises a suspension of particles in a fluid carrier.

144. The method of Claim 143, further comprising allowing the fluid carrier to dissipate thereby depositing the particles at the first region of the article surface.

145. The method of Claim 140, wherein the fluid precursor comprises a chemically active agent in a fluid carrier.

146. The method of Claim 145, further comprising allowing the fluid carrier to dissipate thereby depositing the chemically active agent at the first region of the article surface.

147. The method of Claim 145, wherein the chemically active agent comprises a polymer precursor.

148. The method of Claim 147, further comprising forming a polymeric article from the polymer precursor.

149. The method of Claim 145, wherein the chemically active agent comprises an agent capable of promoting deposition of a material.

150. The method of Claim 145, wherein the chemically active agent comprises an etchant.

151. The method of Claim 150, further comprising allowing the second portion of the surface of the article to be etched.

152. The method of Claim 125, further comprising removing the elastomeric mask of the masking system from the first portion of the article surface

while leaving the agent adhered to the second portion of the article surface.

153. A method of delivering a therapeutic agent to a target, the method comprising:

(a) providing a particle produced by a method comprising

(i) disposing a volume of liquid material between two surface elements, wherein at least one of the two surface elements comprises a patterned area;

(ii) contacting the two surface elements by applying contact pressure to create a point of contact between the two surface elements and confine the liquid material to the patterned area of at least one surface element;

(iii) forming at least one or more particles within the patterned area of at least one of the surface elements; and

(iv) releasing the particles from the patterned area of at least one of the surface elements.

(b) admixing the therapeutic agent with the particle; and

(c) delivering the particle comprising the therapeutic agent to the target.

154. The method of Claim 153, wherein the therapeutic agent is selected from one of a drug and genetic material.

155. The method of Claim 154, wherein the therapeutic agent comprises a drug.

156. The method of Claim 154, wherein the genetic material is selected from the group consisting of a non-viral gene vector, DNA, RNA, RNAi, and a viral particle.

157. The method of Claim 153, wherein the particle comprises a biodegradable polymer.

158. The method of Claim 157, wherein the biodegradable polymer is selected from the group consisting of a polyester, a polyanhydride, a polyamide, a phosphorous-based polymer, a poly(cyanoacrylate), a polyurethane, a polyorthoester, a polydihydropyran, and a polyacetal.

159. The method of Claim 158, wherein the polyester is selected from the group consisting of polylactic acid, polyglycolic acid, poly(hydroxybutyrate), poly(ε-caprolactone), poly(β-malic acid), and poly(dioxanones).

160. The method of Claim 158, wherein the polyanhydride is selected from the group consisting of poly(sebacic acid), poly(adipic acid), and poly(terpthalic acid).

161. The method of Claim 158, wherein the polyamide is selected from the group consisting of poly(imino carbonates) and polyaminoacids.

162. The method of Claim 158, wherein the phosphorous-based polymer is selected from the group consisting of polyphosphates, polyphosphonates, and polyphosphazenes.

163. The method of Claim 157, wherein the biodegradable polymer further comprises a polymer that is responsive to a stimulus.

164. The method of Claim 163, wherein the stimulus is selected from the group consisting of pH, light, and temperature.

165. The method of Claim 153, wherein the particle has a diameter less than 100 microns.

166. The method of Claim 153, wherein the particle has a diameter less than 10 microns.

167. The method of Claim 153, wherein the particle has a diameter less than 1 micron.

168. The method of Claim 153, wherein the particle has a diameter less than 100 nm.

169. The method of Claim 153, wherein the particle has a diameter less than 10 nm.

ABSTRACT OF THE DISCLOSURE

The presently disclosed subject matter describes the use of fluorinated elastomer-based materials, in particular perfluoropolyether (PFPE)-based materials, in high-resolution soft or imprint lithographic applications, such as micro- and nanoscale replica molding, and the first nano-contact molding of organic materials to generate high fidelity features using an elastomeric mold. Accordingly, the presently disclosed subject matter describes a method for producing free-standing, isolated nanostructures of any shape using soft or imprint lithography techniques.